Early DNA Synthesis in the X-chromosome of *Pellia neesiana*

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Introduction

It has been reported that the DNA synthesis in heterochromatins of animals and higher plants occurs at different stages in comparison with that in euchromatins (Lima-de-Faria 1959, Morishima *et al.* 1962, Hsu *et al.* 1964, Tanaka 1965 and others). There is, however, no report on the pattern of DNA synthesis in the heterochromatin of Bryophyta. It has been known in many species of Bryophyta that most portion of their sex-chromosomes are composed of heterochromatin and show strongly condensed heteropycnosis (Heitz 1928, Tatuno 1941, Tatuno and Watanabe 1952, Segawa 1965). The present investigation was made on the X-chromosome of *Pellia neesiana* ($n=9$), Hepaticae, for the analysis of the pattern of DNA synthesis in both heterochromatin and euchromatin by the use of H^3 -thymidine autoradiography.

Material and method

The female gametophytes of *Pellia neesiana* (Gottsche) Limpricht were obtained from Hiroshima, Japan. About 300 thalli were cultivated in glass dishes of 15 cm in diameter. They were planted on sanitary cotton moistened with tap-water and left in a refrigerator at a temperature ranging from 16°~18°C illuminated by a fluorescence light. The thalli elongated at about 0.5~1.0 cm were used for the present investigation. The use of H^3 -thymidine treatment was as follows: Thalli were immersed in H^3 -thymidine (TRA. 61, thymidine-6-T(n), Batch 41, obtained from the Japan Radiochemical Centre) solution diluted with 1/2 Knop's solution at 17°C for 2 hours. The specific activity of H^3 -thymidine used was 20 μ c/ml. After labelling with H^3 -thymidine, the thalli were washed thoroughly in a nonisotopic solution containing an excess of unlabelled thymidine (50 μ g/ml) diluted with 1/2 Knop's solution. Then, the thalli were placed on a cotton moistened with a nonisotopic solution containing an excess of unlabelled thymidine (25 μ g/ml) diluted with 1/2 Knop's solution. They were collected at various intervals (2, 4, 6, 8, 10, 14, 18, 22, 26, 28, 32, 38, 40, 44, 49 hours) from the beginning of the label treatment, and fixed in a modified Carnoy's solution, ethyl alcohol (3): acetic acid (1): chloroform (1) at 10°C. They were hydrolyzed for 13 minutes in

1N-HCl at 60°C and stained with Feulgen's nuclear reaction. For the preparation of autoradiography a method similar to the previous report (Tanaka 1965) was used. After the removal of the cover glass by dry ice method, Sakura stripping film NR-M2 obtained from the Sakura Photoindustrial Co. was applied on the squashed material. The usual squash preparation using Feulgen's stain or aceto-orcein stain were also made in order to observe the morphology of the interphase nuclei and the mitotic chromosomes. Materials fixed in ethyl alcohol (3) : acetic-acid (1) : chloroform (1) were stained with a few drops of hydro-aceto-orcein, a mixture of 9 parts of 2% aceto-orcein and 1 part of 1N-HCl.

Results

Interphase nuclei

It was observed that the interphase nuclei in the meristematic regions showed a large variation in the diameter of the nuclei and in the morphology of the heterochromatic bodies. The interphase nuclei could be divided into four types of nuclei from their size and the morphology of the hetero-

Fig. 1. Photomicrographs and drawings of interphase nuclei in the female gametophyte of *Pellia neesiana* (n=9). a, b, two daughter nuclei immediately after telophase; c, d, diffused nuclei; e, f, partly condensed nuclei; g, h, fully condensed nuclei. $\times 1500$.

chromatic bodies: 1) daughter nuclei in the cells fixed immediately after telophase were small in their diameter and had several small heterochromatic bodies (Fig. 1a and b); 2) nuclei which were medium in size and considered

to be in the middle interphase stage showed diffused chromatine (Fig. 1*c* and *d*); 3) nuclei with a partly condensed and round heterochromatic body (Fig. 1*e* and *f*); 4) nuclei which were the largest in size and considered to be in the late interphase stage had a large and fully condensed club-shaped heterochromatic body (Fig. 1*g* and *h*).

By the use of H^3 -thymidine, the pattern of DNA synthesis in the four types of nuclei were investigated. In the preparations fixed immediately after the 2 hour treatment with H^3 -thymidine, activated silver grains were found only in the second and the third type interphase nuclei which either had diffused chromatin or partly condensed heteropycnotic bodies. The findings indicate that among the four types of interphase nuclei only these two types synthesize DNA, thus indicating that these two are the nuclei at DNA

Fig. 2. Autoradiograms of interphase nuclei fixed immediately after the H^3 -thymidine pulse treatment. *a*, diffused nucleus; *b*, partly condensed nucleus which has activated silver grains over the partly condensed heteropycnotic body; *c*, partly condensed nucleus which has no activated silver grains over the partly condensed heteropycnotic body. $\times 1500$.

synthetic phase (S phase). A name "diffused nuclei" will be given to the former and "partly condensed nuclei" to the latter.

In most diffused nuclei activated silver grains were usually found scattered over all of their nuclear regions, while in some diffused nuclei the activated silver grains were found localized in a limited region (Fig. 2*a*). In partly condensed nuclei activated silver grains were always observed to scatter over all of their nuclear regions including the condensed heteropycnotic body. The activated silver grains on the condensed heteropycnotic body were found to situate either on the whole regions of the condensed heteropycnotic body (Fig. 2*b*) or only on the peripheral regions of the condensed body (Fig. 2*c*).

Mitotic chromosomes

Labelling of mitotic chromosomes was observed for the first time in the cells at 8 hour from the beginning of H^3 -thymidine treatment. The chromosomes at this time had activated silver grains situated mainly on the autosomes which have euchromatin in most regions of both chromosome arms.

X-chromosome of this species was observed to have three large hetero-

chromatic segments: whole short arm, the proximal and the distal region of the long arm. In addition to the heterochromatic segment, the X-chromosome possessed a small euchromatic segment in the middle region of the long arm (Figs. 3a and 4a). The same observation was reported by Heitz (1928), Tatuno (1941), Tatuno and Watanabe (1952) and Segawa (1965).

X-chromosomes in the cells fixed at 8 hour from the beginning of H^3 -thymidine treatment were observed to have activated silver grains only on the euchromatic segment. While the heterochromatic segments had no activated silver grain (Figs. 3b and 4b).

Fig. 3. Photomicrographs and autoradiograms of somatic chromosomes in the female gametophyte of *Pellia neesiana* ($n=9$). Arrows indicate the X-chromosomes. a, photomicrograph of the prometaphase chromosomes stained with aceto-orcein; b, c, and d, autoradiograms of the prometaphase chromosomes fixed at 8 hr., prophase chromosomes fixed at 14 hr., and prometaphase chromosomes fixed at 22 hr., respectively, from the beginning of H^3 -thymidine pulse treatment. $\times 1500$.

The same labelling pattern with the X-chromosome in the cells fixed at 8 hour was found in the cells fixed at 10 hour from the beginning of H^3 -thymidine treatment.

The first appearance of the activated silver grains on the heterochromatic segments of X-chromosomes was observed at 14 hour from the beginning of H^3 -thymidine treatment. In these cells both X-chromosomes and autosomes were found having activated silver grains on their whole regions (Fig. 3c).

In the cells fixed at 18 hour from the beginning of H^3 -thymidine treatment, it was also observed that there was no difference in the labelling pattern between the X-chromosome and the autosomes.

In the cells fixed later than 22 hour from the beginning of H^3 -thymidine treatment, the heterochromatic segments of X-chromosomes were observed having more activated silver grains than the euchromatic segments (Figs. 3d and 4d). In these cells autosomes were also found having few grains.

Discussion

The heterochromatin in the X-chromosome of *Pellia neesiana* was observed to incorporate H^3 -thymidine only during the early and the middle stages of S phase, while the euchromatin in the same X-chromosome was observed to incorporate H^3 -thymidine continuously from the early to the last stages of S phase. During the early stages of S phase the heterochromatin was observed to incorporate more H^3 -thymidine than the euchromatin. These observations indicate that DNA in the heterochromatin of X-chromosome replicates mainly during the early and the middle stages of S phase and finishes its replication earlier than the DNA in euchromatin.

It was reported that the DNA replication in heterochromatin is either later (Mori-shima *et al.* 1962, and others) or earlier (Hsu *et al.* 1964, Tanaka 1965) in comparison with the DNA replication in euchromatin. In the present investigation an additional early replicating heterochro-

Fig. 4. Autoradiograms and schematic drawings of the X-chromosomes in the female gametophyte of *Pellia neesiana* fixed at 2 hr., 8 hr. and 22 hr., respectively, from the beginning of the H^3 -thymidine pulse treatment. $\times 2500$.

matin was found in the X-chromosome of a Bryophyta species *Pellia neesiana*.

Difference in the euchromatin within one chromosome was previously found in the autosomes of some species of higher plants (Lima-de-Faria 1959, Tanaka 1965, 1966). In the present material, however, the same difference was found for the first time in the sex-chromosome.

Interphase nuclei during S phase were divided into two types through their morphological characteristics: diffused nuclei and partly condensed nuclei. It is presumed that the diffused nuclei are those at the early S phase and the partly condensed nuclei are those at the middle and late S phase, because the former was found to be smaller in size in comparison with the latter. In the

diffused nuclei the DNA synthetic sites were found to occur either scattered over the nuclei or localized in a restricted region of the nuclei. In partly condensed nuclei that DNA synthetic sites were found to occur scattered over the whole regions of the nuclei. In these nuclei most of the heteropycnotic bodies, however, were found having DNA synthetic sites in their peripheral regions and a few of them in their whole regions. In addition to these observations DNA replication in the heterochromatin of the present X-chromosome was found to start at the early stage of S phase. These observations will indicate that the DNA replication in the heterochromatin of the present X-chromosome starts during the diffused state of heterochromatin in the diffused nuclei. Presumably, after the completion of its DNA replication the heterochromatin may come together in one place forming a partly condensed heteropycnotic body. Similar observations have been presented in the autosomes of *Spiranthes sinensis* (Tanaka 1965).

Summary

In the present investigation DNA synthesis of X-chromosome of *Pellia neesiana* ($n=9$), Hepaticae, was studied by the use of the H^3 -thymidine autoradiography.

1. DNA in the heterochromatin of X-chromosome of female *Pellia neesiana* was found to replicate during the early and the middle stages of DNA synthetic phase.

2. DNA in the euchromatin of both X-chromosome and autosomes was found to replicate continuously from the early to the last stages of DNA synthetic phase.

3. DNA in the heterochromatin of X-chromosome was found to replicate mainly in the diffused state of heterochromatin during the early stage of DNA synthetic phase.

Literature cited

- Heitz, E. 1928. Das Heterochromatin der Moose. I. Jahrb. wiss. Bot. 69: 761-818.
- Hsu, T. C., Schmid, W. and Stubblefield, E. 1964. DNA replication sequences in higher animals. In *The Role of Chromosomes in Development*. 83-112.
- Lima-de-Faria, A. 1959. Differential uptake of tritiated thymidine into hetero- and euchromatin in *Melanoplus* and *Secale*. J. Biophys. Biochem. Cytol. 6: 457-466.
- Morishima, A., Grumbach, M. N. and Taylor, J. H. 1962. Asynchronous duplication of human chromosomes and the origin of sex chromatin. Proc. Natl. Acad. Sci. U.S. 48: 756-763.
- Segawa, M. 1965. Karyological studies in liverworts, with special reference to structural sex chromosomes. III. J. Sci. Hiroshima Univ. Ser. B. Div. 2. 10: 149-178.
- Tanaka, R. 1965. H^3 -thymidine autoradiographic studies on the heteropycnosis, heterochromatin and euchromatin in *Spiranthes sinensis*. Bot. Mag. Tokyo 78: 50-62.
- 1966. DNA replication in *Chrysanthemum lineare*, *Ch. nipponicum* and their F_1 hybrid. ibid. 79: 447-456.
- Tatuno, S. 1941. Zytologische Untersuchungen über die Lebermoose von Japan. J. Sci.

- Hiroshima Univ. Ser. B. Div. 2. 4: 73-187.
- and Watanabe, K. 1952. Cytological studies on the *Pellia Neesiana* with aceto-orcein, with special reference to the chromonema. Jap. Jour. Genet. 27: 28-32.
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