Asynchronous DNA Replication in the Chromosomes of *Haplopappus gracilis* ($2n=4$)¹

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The discovery of the chromosome number ($2n=4$) of *Haplopappus gracilis* (Jackson 1957) stimulated the study of basic karyotype theory which resulted in evidence for the localized kinetochore producing the V- and J-shaped chromosomes and for the secondary constriction producing satellites. These are the basic morphological characteristics of the karyotype for this species. According to the observation of Jackson (1962), the individual chromosomes were found to consist of segments, each of which was homologous to the chromosomes of its related species *H. ravenii* ($2n=8$). A similar observation was presented by the present author through the study of heterochromatic segments in somatic prophase chromosomes (Tanaka 1967). This indicated that there is a complex organization of the chromosomes of this species. In the present investigation a H^3 -thymidine autoradiographic study was done in order to elucidate the distribution of DNA replicating sites in the chromosomes of this species.

Materials and methods

An inbreeding experiment was continued for five generations on *Haplopappus gracilis*, (R. Jackson's Collection No. 2691-11) to select a strain being resistant of fungus disease in hypocotyl. The name 'Kansas-Hiroshima Strain No. 1' was given to the selected strain.

Five clones of the strain were cultivated in 0.02% Hyponex solution (Hyponex Chem. Co., Ltd., U.S.A.) at 20°C for 10 days. The new roots developed from the clones were treated with 2 μ C/ml of H^3 -thymidine solution diluted in 0.02% Hyponex solution for 0.5 hr at 20°C (pulse label). The H^3 -thymidine was TRA. 61, Thymine-6-T(n), Batch 34 obtained from the Japan Radiochemical Centre. The roots of clones were washed thoroughly in water after treatment with H^3 -thymidine, and were returned to the Hyponex solution. Root tips were fixed in acetic alcohol (1:3) at 15°C. Six root tips were fixed at each of the following times after the beginning of H^3 -thymidine treatment: 0.5 hr, 2.0 hr, 2.5 hr, 3.0 hr, 3.5 hr, 4.0 hr, 6.0 hr, 8.0 hr, 10.0 hr and 14.0 hr. Procedures similar to those described in earlier papers (Tanaka 1965, 1966) were applied to the present autoradiographic treatment. Chromosomes were stained in Feulgen's nuclear reaction (hydrolyzed in 1 N HCl at 60°C for 8 min). For the autoradiography, Sakura stripping film NR M2 (Konishiroku Photo Ind. Co., Ltd., Japan) was applied to squash preparations.

Observations

1. Nuclei just after the 0.5 hr pulse treatment with H^3 -thymidine

About half of interphase nuclei were labelled. In all of the labelled interphase nuclei, non-labelled heteropycnotic bodies were observed. This

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finding indicates that the heterochromatins of this species do not synthesize DNA during condensation, as was found in *Spiranthes sinensis* (Tanaka 1965). However, DNA synthesis in heterochromatins was found to occur during their uncontracted state, because the heteropycnotic bodies appeared with heavy label at fixation times later than 2.0 hrs.

The labelling pattern of interphase nuclei was not homogeneous. In most of the interphase nuclei, labelled regions were found forming the clusters of silver grains. The number of clusters of labelled regions in a nucleus varied from one to four. Most nuclei showed four blocks of labelled regions (Fig. 1a), which were comparable with the chromosome number of the present species. Each of the blocks of labelled regions might indicate one individual chromosome in the interphase nucleus.

2. Duration of G_2 and S phases

The first appearance of labelled prophase nuclei was detected in the preparation fixed at 2.5 hr from the beginning of H^3 -thymidine treatment. The proportion of labelled prophase nuclei at the fixation time of 2.5 hr was very low, varying from about 10% to 30% of prophase nuclei.

Fig. 1. Autoradiographs of the root tip cells of *Haplopappus gracilis*, $2n=4$. $\times 1600$.
a, two interphase nuclei fixed just after the 0.5 hr pulse treatment with H^3 -thymidine.
b, a prophase chromosome complement fixed at 10 hr from the beginning of pulse treatment with H^3 -thymidine.

At a fixation time of 3.0 hr, about half of the prophase nuclei were observed to have labelled chromosomes. The duration of the G_2 phase, therefore, can be estimated to be 3.0 hrs, which is comparable to the G_2 duration in seedlings, 2.6 hrs, calculated by Ames *et al.* (1966), but about twice as long as that calculated by Sparvoli *et al.* (1966).

At fixation times of 3.5 hr and 4.0 hr, the proportion of labelled prophase nuclei increased, reaching a maximum value of 95% of prophase nuclei. In all of the preparations fixed at a fixation time of 6.0 hr, most of the prophase nuclei were observed to be labelled. The first indication of a decrease in the proportion of labelled prophase nuclei was found at a fixation time of 8 hr, with the proportion 85%. It was 55% at 10 hr and 40% at 14 hr. Thus, the duration of the S phase can be calculated to be $10 \text{ hr} - 3 \text{ hr} = 7 \text{ (hrs)}$ which

was comparable to the S duration in seedlings, 6.8 hrs, calculated by Ames *et al.* (1966), but was again about 1.7 times longer than that calculated by Sparvoli *et al.* (1966).

3. The pattern of DNA synthesis in chromosomes

The diploid chromosomes of this species can be divided into a pair of large V-shaped chromosome (chromosome 1) and a pair of J-shaped and satellited chromosomes (chromosome 2) (cf. Jackson 1957, 1962; Tanaka 1967). Chromosome 1 possesses three large heterochromatic segments located independently in the proximal regions of both arms, in a median region of the long arm and in a subdistal region of the long arm. The chromosome 2 possesses two heterochromatic regions situated in the proximal regions of

Fig. 2. Prophase chromosomes and their autoradiograph in the root tip cells of *Haplopappus gracilis*, $2n=4$, fixed at 2.5 hr from the beginning of pulse treatment with H^3 -thymidine. Letters 1 and 2 indicate chromosomes 1 and 2, respectively. $\times 2400$.

both arms and in the satellite. Details of the karyotype of this species were reported in a previous paper (Tanaka 1967).

Chromosomes in all of the labelled prophase nuclei fixed at both 2.5 hr and 3.0 hr showed a discontinuous labelling which indicated that independent DNA replication in individual segments occurred during the final stage of the S phase. Most of the labelled prophase nuclei collected at subsequent fixation times of 3.5, 4.0 or later hours, showed a continuous labelling of whole regions of chromosomes (Fig. 1b). A few of the prophase nuclei collected at these fixation times, however, showed discontinuous labelling patterns.

Chromosomes labelled at the final stage of S phase were studied morphologically. In chromosome 1, there were three heavily labelled regions, each of which was well localized in the three large heterochromatic regions: in the proximal regions of both arms; in a median region of long arm; and in a

Fig. 3. Prophase chromosomes and their autoradiographs in the root tip cells of *Haplopappus gracilis*, $2n=4$, fixed at 3.0 hr from the beginning of pulse treatment with H^3 -thymidine. Letters 1 and 2 indicate chromosomes 1 and 2, respectively. $\times 2400$.

subdistal region of long arm (Figs. 2 and 3). Both of the homologues of chromosome 1 showed the same pattern of localized labelling for the three heterochromatic regions.

In addition to the heavy labelling of the heterochromatic regions there was found to be light labelling in two sites located in a median region and in a subproximal region of the short arm of chromosome 1. The lightly labelled

regions were observed to be euchromatic. The remaining regions of both arms, all of which were euchromatic, were observed to be completely unlabelled at the end of the S period.

In both homologues of chromosome 2, satellites and the proximal regions of both arms were found to have heavy labelling at the final stage of the S phase. The heavily labelled regions were found to be heterochromatic. Light labelling was observed in a median region of the long arm of chromosome 2. The lightly labelled region was found to be euchromatic. In most nuclei, the light labelling was found to appear in both homologues of chromosome 2. Sporadic labelling was observed in a distal region of the long arm of chromosome 2.

Discussion

According to the observations of Jackson (1962) on meiotic chromosome configurations in the F_1 hybrid of *Haplopappus gracilis* $\times$ *H. ravenii*, chromosome 1 of *H. gracilis* had segments which were genetically homologous to the chromosomes of *H. ravenii* ($2n=8$). Karyotype analysis in the two species (Tanaka 1967) showed that chromosome 1 was composed of the three segments which morphologically resembled three of the four chromosomes of *H. ravenii*. In the present investigation it was found that chromosome 1 had three late-replicating regions located independently in the proximal regions of both arms, in a median region of long arm and in a subdistal region of long arm. Each of the late-replicating regions is considered to be a terminal of DNA replication in chromosome 1. Chromosome 1 is, thus, regarded as a compound chromosome, composed of the three segments each of which replicates independently.

In chromosome 2, there are two late-replicating regions situated in the proximal regions of both arms and in the satellites. The late-replicating regions of both chromosomes 1 and 2 were found to be heterochromatic. Further, the heterochromatic regions and euchromatic regions of both chromosomes showed vigorous DNA replication during the early and middle stages of the S phase. Thus, the duration of DNA replication in the heterochromatin of *H. gracilis* was found to be longer than that of DNA replication in euchromatin.

Résumé

1. Chromosome 1 (V-shaped chromosome) of *Haplopappus gracilis* ($2n=4$) had three late-replicating regions located independently in the proximal regions of both arms, in a median region of the long arm and in a subdistal region of the long arm.

2. Chromosome 2 (J-shaped, satellited chromosome) of this species had two late-replicating regions situated in the proximal regions of both arms and in the satellite.

3. All of the late-replicating regions in the two pairs of chromosomes were found to be heterochromatic.

4. The duration of DNA replication in heterochromatins was found to be longer than that in euchromatins.

5. It was presumed that chromosome 1 was organized as a compound chromosome composed of three independently replicating segments.

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