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The heterochromatin of *Nicotiana otophora*[1]

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Abstract. The haploid complement of *Nicotiana otophora* Grisebach comprises five large acrocentric and seven small metacentric or submetacentric chromosomes. All the large chromosomes have a large block of heterochromatin on the long arm whereas the small chromosomes contain only small pieces of heterochromatin mostly around their centromeres. The replication of chromosomes was investigated by a pulse labeling technique of autoradiography. In samples collected at 4.5 to 6.5 h after pulse labeling, only the five large heterochromatic blocks were labeled. Centromeric heterochromatin was labeled at 7.5 h, and after 9.5 h only euchromatin was labeled. These observations indicate that the large heterochromatic blocks are the last component of the genome to complete DNA replication whereas euchromatin is the earliest; centromeric heterochromatin also replicates late but not as late as the large heterochromatic blocks.

Key words: Heterochromatin; Karyotype; Late replication; *Nicotiana otophora*.

Introduction

Nicotiana otophora Grisebach ($2n=24$) is a wild diploid species closely related to cultivated tetraploid *N. tabacum* ($2n=48$) as F_1 hybrids of these two species generally showed 12 bivalents and 12 univalents at metaphase I (Goodspeed, 1954). However, *N. otophora* possesses ten large blocks of heterochromatin that are not present in *N. tabacum*. Burns (1966) showed that the heterochromatic blocks of *N. otophora* are located on the long arms of five pairs of acrocentric chromosomes, four being terminal and six subterminal. According to the survey of Merritt (1974), *N. otophora* has the most heterochromatin of any species of the genus.

It has been shown that the heterochromatic blocks of *N. otophora*, when introduced into the nucleus of *N. tabacum*, are unstable; they broke spontaneously or enlarged to form megachromosomes (Gerstel and Burns, 1967). Gupta (1969) hypothesized that instability of the chromosomes of *N. plumbaginifolia* in the background of *N. tabacum* is caused by delayed replication of heterochromatin in these chromosomes. In maize, Rhoades and Dempsey (1972) demonstrated that in the presence of two or more B chromosomes the knobbed A chromosomes break frequently during the second pollen mitosis. They interpreted that the breakage is caused by delayed replication

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of the heterochromatic knobs under the influence of the B chromosomes (Rhoades and Dempsey, 1973). Subsequently, Pryor *et al.* (1980) showed that in root-tip cells of maize the heterochromatic knobs are the last component of the genome to complete DNA synthesis. Late replication of heterochromatin has also been shown to be responsible for chromosome breakage in tissue cultures of *Avena sativa* (Johnson, *et al.*, 1987) and maize (Lee and Phillips, 1987).

Recently, we have found that the chromosomes of *N. otophora* bearing a large heterochromatic block can break spontaneously in interspecific somatic hybrids (Chen, unpublished results). In an attempt to understand the mechanism of the breakage, we have firstly studied DNA replication in chromosomes of *N. otophora*. The results of this study are presented herein.

Materials and Methods

Haploid plantlets of *N. otophora* Grisebach obtained from anther culture (Chen *et al.*, 1985) were used as the experimental material. The plantlets were routinely maintained and propagated by culturing lateral buds in a nutrient medium containing the major salts (half strength) of Murashige and Skoog (1962), the minor salts of Heller (1953), 1% sucrose, and 0.8% agar.

For preparation of slides for karyotype analysis the following procedure was followed. Roots of the plantlets were treated with 0.002 M 8-hydroxyquinoline at 18 to 20°C for 3h, fixed in ethanol-acetic acid (3:1) overnight, stained by the Feulgen method, and then treated with 5% pectinase at room temperature for 1 h. Root tips were squashed on slides in 45% acetic acid.

DNA replication in chromosomes was investigated by a conventional technique of autoradiography. Roots of the plantlets were immersed in nutrient solution containing methyl-[^{3}H]thymidine (5 μCi/ml, specific activity 6.7 Ci/mM) for 30 min. After the isotope treatment, plantlets were washed thoroughly in distilled water and then transferred to nutrient solution without the isotope for further growth. Samples of roots were fixed in ethanol-acetic acid (3:1) at various times. To increase the frequency of metaphases and to facilitate chromosome spreading, in some experiments roots were treated with 0.02% colchicine for 2.5 h prior to fixation. Microscopic slides were prepared by the Feulgen squash method. Cover slips were removed by the dry-ice method (Conger and Fairchild, 1953); the slides were dehydrated in 95% ethanol and air dried. Slides were dipped in a 1:1 aqueous dilution of Kodak NTB-2 nuclear track emulsion, exposed at 4°C for 2 weeks, and developed in Kodak D-19 developer at 18°C for 2.5 min.

Results and Discussion

Chromosomal Locations of Heterochromatin

Large, darkly stained heterochromatic blocks were present in all interphase nuclei examined. The maximum number of the blocks in root-tip nuclei of haploid plants was five, thus corresponding to the number of ten observed in diploid plants (Burns, 1966; Merritt, 1974). Burns (1966) and Merritt (1974) reported that the heterochromatic blocks were uniform in size. However, according to our observation, two of these are appreciably larger than the other three (Fig. 1). In addition to the large heterochromatic blocks, small, darkly stained granules were also observed.

At prometaphase, the haploid complement of *N. otophora* was seen as comprising five large and seven small chromosomes (Fig. 2). Of the large chromosomes, two possess a terminal and three possess a subterminal heterochromatic segment. The terminal heterochromatic segments, which represent approximately half of

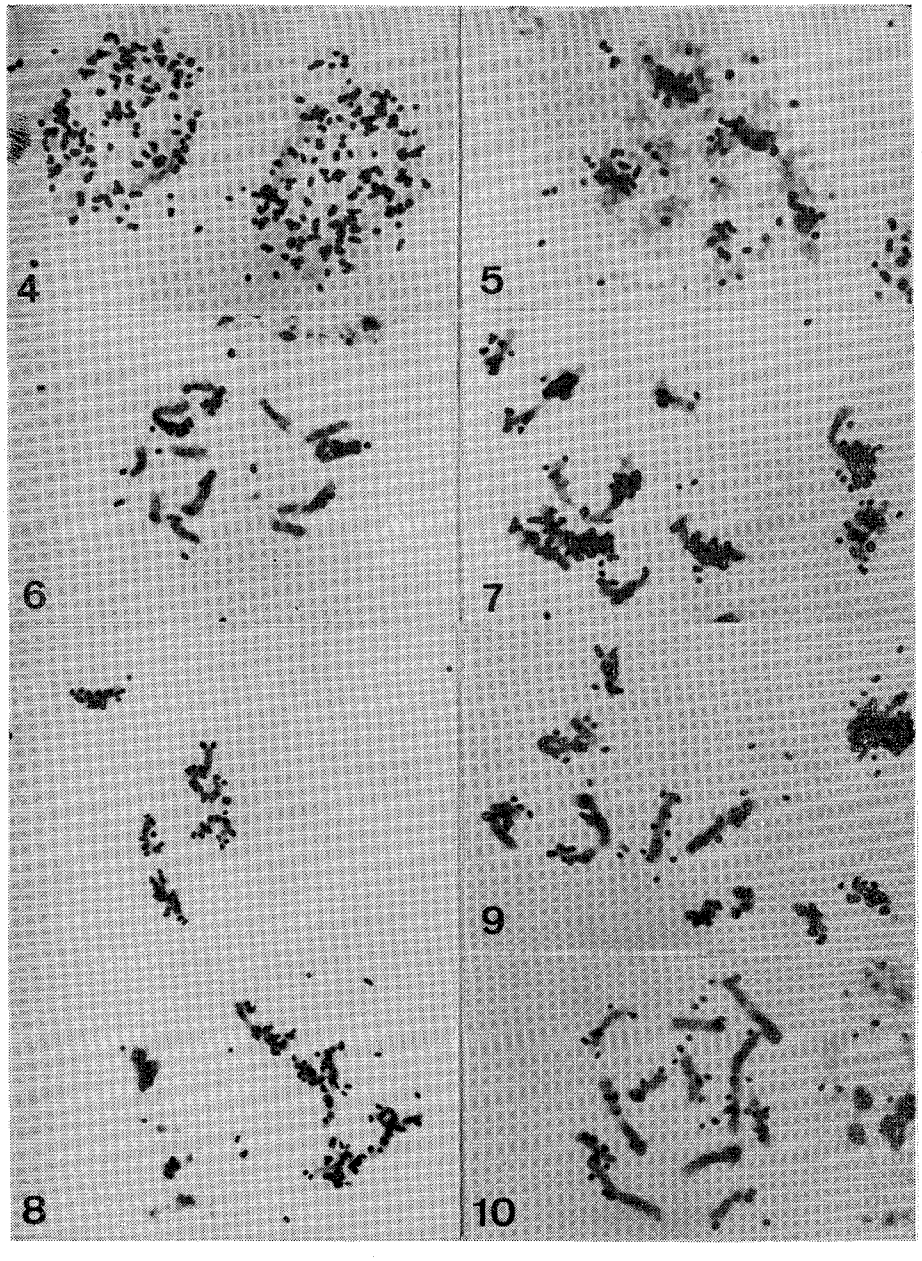
Figs. 1-3. Interphase nuclei and mitotic chromosomes of haploid *Nicotiana otophora*. 1. Two interphase nuclei, each with 5 large blocks of heterochromatin (×1300). 2. Prometaphase showing the large and small heterochromatic segments in different chromsomes (×1500). 3. Metaphase chromosomes showing the centromeres and satellites; arrows indicate the three large subterminal heterochromatic segments (×2400).

the total length of the chromosomes, are longer than the subterminal ones. The small chromosomes contain no large heterochromatic segments, but only small, darkly stained bands around their centromeres.

At metaphase, the centromeres and secondary constrictions became clearly visible. As pointed out by other investigators (Goodspeed, 1954; Burns, 1966), the five large chromosomes are acrocentric and the seven small chromosomes are metacentric or submetacentric. The five large chromosomes are distinguishable by the position of the heterochromatic segment and centromere, and the presence or absence of a satellite (Fig. 3). The two chromosomes with a terminal heterochromatic segment differ in the length of their short arms. Of the chromosomes with a subterminal heterochromatic segment, two have a satellite. In addition, the size of the satellites differs. It is rather difficult to distinguish chromosomes in the small group since they are all small, lack conspicuous heterochromatin, have median to submedian centromeres, and none possesses a satellite. With respect to the satellited chromosomes, our observation seems at variance with that of Goodspeed (1954) and Burns (1966), who reported that one of the small chromosomes had a satellite.

Chromosome Replication

Two classes of labeled interphase nuclei were observed immediately after treatment with [^{3}H]-thymidine, one (83.9%) with silver grains distributed over the whole nucleus (Fig. 4) and the other (16.1%) with silver grains clustered at a few spots in the nucleus (Fig. 5). For convenience of description, these will be referred as "whole ladel" and "spot label", respectively. The presence of these two classes of labeled interphases indicates that DNA replication in chromosomes of *N. otophora* is asynchronous. The number of labeled spots in the latter class of nuclei varied, but five appeared to be maximum. Thus, the number of labeled spots corresponds to the number of large heterochromatic blocks in the nucleus, suggesting a causal relationship between the large heterochromatic blocks and asynchrony of DNA replication.



Figs. 4-10. Distribution of label in interphase nuclei and on prometaphase chromosomes of *Nicotiana otophora* at the sequential times of fixation after treatment with [^{3}H]-thymidine. 4. Silver grains distributed over the whole nucleus, immediately after the treatment (×1500). 5. Silver grains clustered at five spots in the nucleus, immediately after the treatment (×1500). 6. Silver grains restricted to the large heterochromatic segments, 6.5 h after the treatment (×1300). 7. Silver grains on the large heterochromatic segments and in the centromeric regions, 7.5 h after the treatment (×1400). 8. Silver grains distributed along the entire length of all chromosomes, 8.5 h after the treatment (×1250). 9. Silver grains in the euchromatic regions, 9.5 h after the tratment (×1500). 10. Silver grains in the euchromatic regions, 10.5h after the treatment (×1500).

Silver grains were first observed over prometaphases at 4.5 h after the isotope treatment. At this time only the large heterochromatic segments of the acrocentric chromosomes were labeled; other regions of the genome were devoid of silver grains (Fig. 6). This pattern of label continued to appear during the next two hours of growth. By the time of 7.5 h after the treatment, however, in addition to the large heterochromatic segments, the centromeric regions of most chromosomes were also labeled (Fig. 7). Silver grains were distributed along the entire length of all chromosomes (Fig. 8) at 8.5 h. From 9.5 to 11.5 h, only the euchromatic regions were labeled (Figs. 9 and 10). Label in the euchromatic regions was the heaviest at 8.5 h and became lighter thereafter. No labeled prometaphases were observed at 12.5 h.

Observations on distribution of label over prometaphases indicate that the cytologically different components of the chromosomes of *N. otophora* are also different with respect to the time of DNA replication in the S period. Because after the [^{3}H]-thymidine treatment silver grains first appeared on the large heterochromatic segments long before other regions were labeled, the large heterochromatic segments must be the last component of the genome to complete DNA synthesis. No evidence has been found for differential replication among the large heterochromatic segments. The percentage of labeled interphases with the spot label pattern (16.1%) immediately after the treatment provides a measure of the proportion of the S period in which late replication occurs. Centromeric heterochromatin is also late in replication when compared with euchromatin. Thus, the patterns of chromosome replication in *N. otophora* are similar to those observed by Pryor *et al.* (1980) in maize. These investigators found that the different classes of heterochromatin in maize have their own periods of DNA replication during the S period, knob heterochromatin being the last to complete replication.

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野生菸草 *Nicotiana otophora* 的異染色質

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單倍體菸草 *Nicotiana otophora* Grisebach 具有五條較長的近端中節及七條較短的中央中節或次中央中節的染色體。每一條較長的染色體的長臂上均有一大塊異染色質，而較短的染色體僅中節附近有小塊的異染色質。以氚一胸腺嘧啶核苷短時間標記根尖染色體的 DNA，然後於不同生長時間固定，做自動放射顯影。發現生長 4.5 至 6.5 小時的材料，僅大塊異染色質處有標記；生長 7.5 小時的材料，各染色體中節兩側之小塊異染色質處有標記；而在生長 9.5 小時後，標記僅出現在眞染色質上。因此知道大塊異染色質是最晚複製的，而眞染色質則最早複製；中節兩側的小塊異染色質亦屬晚複製，但不若大塊異染色質那麼遲。