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DIFFERENTIAL LABELLING OF *TRILLIUM* CHROMOSOMES BY H^3-THYMIDINE AT LOW TEMPERATURE

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1. INTRODUCTION

In some plants growth at low temperature reveals a linear differentiation in the metaphase chromosomes. Depending on the species, temperatures ranging from $-1°$ C. to $+5°$ C., produce some weakly Feulgen positive segments along with other fully positive segments. These segments are constant in relative length and position. The weakly stained segments correspond to the condensed heterochromatic segments seen during the telophase and resting stage nuclei of these species.

In originally describing these observations Darlington and La Cour put forward the hypothesis of nucleic acid starvation, to explain the effect. According to their view the low temperature retards the synthesis of deoxyribose nucleic acid in the nuclei. As a result of this the chromosomes enter mitosis with euchromatic and heterochromatic regions competing for a depleted supply of DNA or its precursors. This view is incompatible with the widely accepted current concept that the amount of DNA per haploid chromosome set is constant.

In a recent study, La Cour *et al.* (1956) have made quantitative measurements of Feulgen staining photo-metrically, using a scanning densitometer devised by Deeley (1955) giving an accuracy of the order of ± 3 per cent. Their measurements were carried out on several plant species with or without visible heterochromatin grown at normal as well as low temperature. They found that :

(1) the plants with as well as without heterochromatin gave smaller values of 2C and 4C for cells at all stages of mitosis when grown at low temperature compared to the controls ;
(2) resting nuclei of the differentiated zone gave reduced values like those in the root meristems ;
(3) cold treated cells which were about to enter mitosis as well as those of the differentiated region returned to normality when brought back to the control temperature ;
(4) a high temperature of the order of 36° C. caused a reduction of the value of C and this reduction was comparable to that caused by low temperature.

According to the authors the simplest explanation for these findings was to assume a true reduction of DNA in cold-treated cells. However,

they also point out that unless proof is available for this view one should keep in mind an alternative explanation, namely, that the change in temperature might alter the reactivity of the DNA to the Feulgen stain.

The present work was undertaken to explore the possibility of gaining information by the application of tracer technique.

2. MATERIAL AND EXPERIMENTAL METHOD

A clone of *Trillium erectum* growing in pots in the green house at the Brookhaven National Laboratory was used. The chromosome complement in this species ($2n = 10$) has several chromosomes with large terminal or intercalary heterochromatic segments. Tritium-labelled thymidine was used to label the DNA of the root meristem cells.

The plants were carefully depotted, their roots washed free of soil and immersed in H^3-thymidine solution having a concentration of 2 μc./ml. and specific activity 1·9 C/m. mole for 16 hours at 22° C. The solution was constantly aerated during the treatment. After the treatment the roots were washed in several changes of water for about ten minutes and the plants repotted in vermiculite taking care to avoid mechanical injury to the roots. The plants so treated were allowed to grow at 22° C. for a period of 24 hours and then kept for 5 days in a refrigerator maintained at 1° C. and lighted constantly with a fluorescent table lamp. After this the root tips were quickly removed from the plants and fixed in La Cour's 2BD (Darlington and La Cour, 1962) for over-night prior to staining by the Feulgen-squash method.

The average range of tritium beta particles in tissues is something of the order of 1 micron only (Hughes *et al.*, 1958) and each effective beta particle affects only one or two silver grains in photographic emulsion applied over the tissue (Herz, 1959). For this reason it was very important to squash the root meristems in such a way as to avoid the overlapping of cells, and to bring about maximum flattening of the chromosomes. This was attained by the application of sufficient pressure on and repeated heating of the slides. This ensured a direct contact of the whole chromosome body with the emulsion. The slides so made, were dipped in Kodak N.T.B. liquid emulsion at 40° C. and stored in the dark in a refrigerator for exposure for a period of three weeks, developed and made permanent in the usual way.

3. RESULTS AND DISCUSSION

The results in this study are based on the observations made on metaphases in twenty root meristems obtained from five plants (four from each). Over 150 metaphases were scored. The pattern of labelling in every case was essentially the same. This is illustrated by figs. 1 and 2 with corresponding autoradiographs. Several heterochromatic segments can be easily distinguished in plate figs. 1*a* and 2*a* by their conspicuously feeble staining as compared to the deep staining euchromatic segments of the chromosomes. In the corresponding autoradiographs as illustrated by plate figs. 1*b* and 2*b* it is clearly seen that the heterochromatic regions are practically devoid of labelling while the euchromatic regions are very heavily labelled. This means that growth at low temperature has succeeded in bringing about a near-absence of DNA from the heterochromatic segments of the chromosomes but has not affected the DNA charge of the euchromatic regions.

Many anaphases and telophases were found to have bridges and fragments induced by the internal irradiation of the cells as has been described by Wimber (1959) for *Tradescantia* and Natrajan (1961) for *Vicia faba* using more or less the same concentration of tritium. Cells were also found which possessed a major nucleus along with usually one but sometimes up to three micronuclei. Without exception the major as well as the micronucleus in these cells was " hot ". These points will be described and discussed elsewhere.

It is common knowledge that a low temperature of this degree and duration considerably stretches the length of interphase period by slowing down the mitotic process in addition to interfering with the process of spindle formation. As a result, in treated materials, there is always an abundance of metaphases. The control slides conformed to this expectation but the tritium treated meristems contained a markedly reduced number of metaphases. No doubt this was the result of mitotic inhibition caused by beta exposure.

The irradiation can induce mitotic inhibition by suppressing DNA synthesis either in cells which are in the presynthesis period or those which have already entered the synthesis period or both. Howard and Pelc (1952) working with *Vicia faba* have shown that cells which are in the period of synthesis or nearly so at the time of X-irradiation are not affected as regards DNA synthesis. They have also shown that cells in the presynthesis period at the time of irradiation are delayed as regards the synthesis. It may further be stated that in the ordinary mitotic cycle DNA synthesis, once started, is always carried on until every chromosome in the nucleus has exactly doubled its DNA content (Pätau and Das, 1961). The only well established exception to this rule concerns the giant chromosomes of *Diptera* (Ficq and Pavan, 1957). However, the Dipteran giant chromosomes cannot be treated on par with the ordinary mitotic chromosomes. These points are very relevant in regard to the cause of the differential labelling of eu- and heterochromatic segments as observed in the present experiment.

Tritium was made available to cells for a period of 16 hours at normal growth temperature. The period of synthesis for *Vicia faba* which contains heterochromatin is of the order of 6 hours and that for *Tradescantia paludosa* without any heterochromatin about 10 hours (Howard and Pelc, 1952 ; Wimber, 1959). It is therefore likely that the period of synthesis in *Trillium erectum* was well covered by the time span of 16 hours. It is also possible that the DNA synthesis, as in the grasshopper *Melanopus differentialis*, is later in the heterochromatin than the euchromatin (Lima-de-Faria, 1959). Thus in those *Trillium* cells which had just started synthesis when H^3-thymidine was fed, the DNA of eu- as well as heterochromatic segments should be found equally labelled at metaphase. Further in those cells where the synthesis was reaching the end in euchromatin but was going on

in heterochromatin, the heterochromatic DNA of metaphase chromosomes would be more heavily labelled.

The observations, however, are not in conformity with either of these expectations : none of the heterochromatic segments during metaphase showed any appreciable label. Thus low temperature has in some way prevented the assimilation of DNA in the heterochromatic segments. It is significant that La Cour *et al.* (1956) found that the values for DNA in the nuclei of materials with as well as without visible heterochromatin ranged from below 2C to below 4C values at low as well as abnormally high temperatures. From the present work it is clear that in a species with heterochromatin this reduction occurs mainly and selectively in the heterochromatic regions. Why this is so, is not clear. The experiment, however, does show that the temperature treatment brings about a true loss of the preformed DNA.

4. SUMMARY

Root meristems of *Trillium erectum* were treated with a solution containing 2 μc./ml. H^3-thymidine for 16 hours at 22° C. Following this treatment the plants were allowed to grow at this temperature for a period of 24 hours and finally kept at 1° C. for 5 days. Slides were made from these plants for autoradiographic study of the chromosomes. The autoradiographs of metaphases showed the euchromatic chromosomal segments to be fully labelled and the " starved " heterochromatic regions to be devoid of the label. Thus the low temperature has induced cytologically observable differentiation between the eu- and heterochromatic segments of the chromosomes which is due to a true and selective loss of the DNA from the heterochromatic regions.

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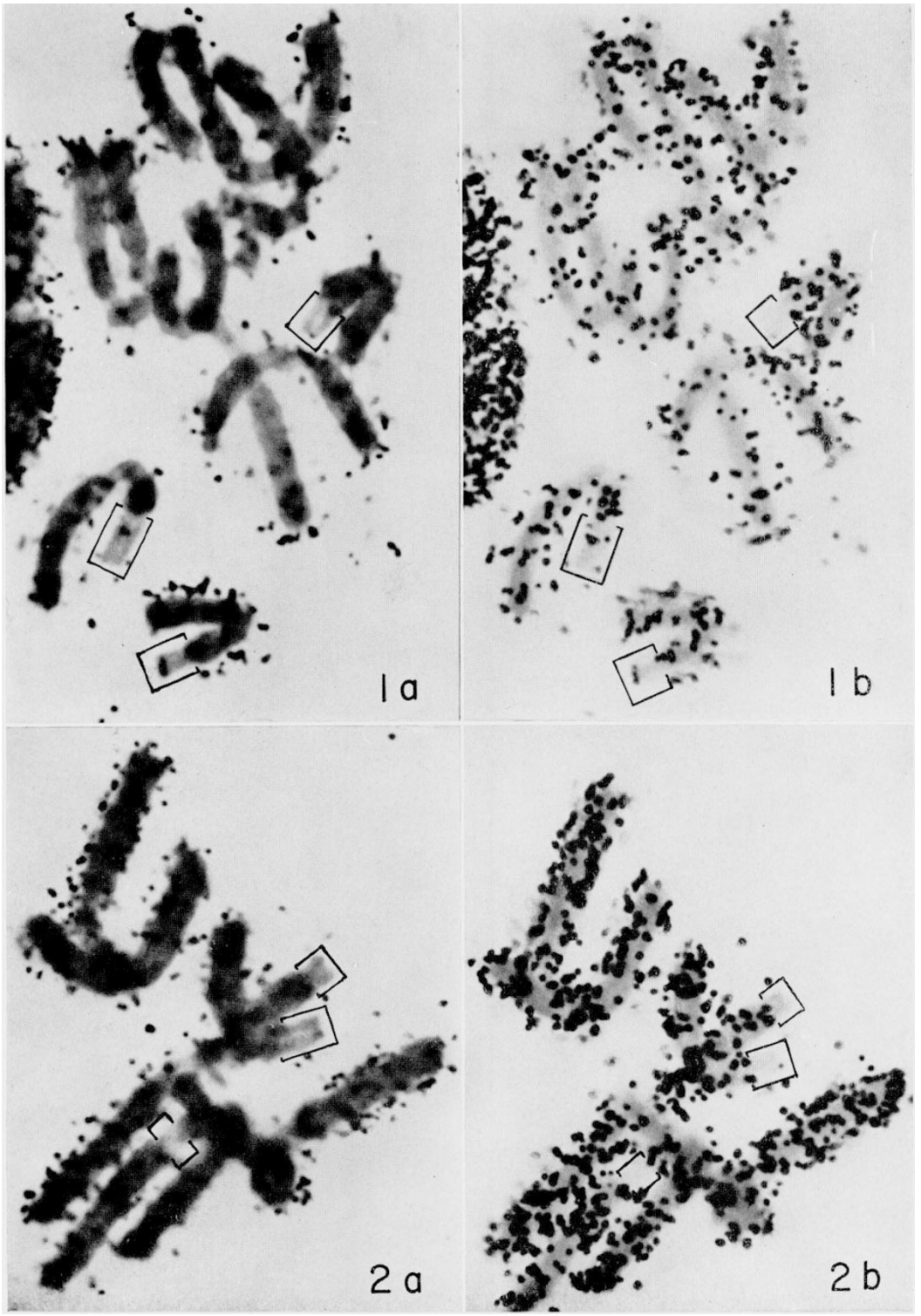

Plates 1a and 2a.—Photomicrographs of metaphases in *Trillium erectum* showing the eu- and the heterochromatic regions in chromosomes.

Plates 1b and 2b.—Photomicrographs of the corresponding autoradiographs showing heavily labelled euchromatic segments and unlabelled heterochromatic segments.

Heterochromatic segments have been demarcated in every figure. ×2000.

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