NOTE

DNA Synthesis during Premitotic (G_2) and Mitotic (M) Phases in the Meristematic Cells of Barley

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Introduction

Recently, the studies of Matsudaira, et al.¹⁾ showed the incorporation of tritiated thymidine triphosphate into acid-soluble fraction of the nuclei isolated from *in vivo*-irradiated and unirradiated Ehrlich ascites tumor cells.

This study was undertaken to investigate the incorporation of ^3H -thymidine triphosphate into nuclei of barley leaf meristem cells. We also report here a cytological evidence of some degree of DNA synthesis during G_2 stage and mitotic phase in the meristematic cells of barley first leaf by autoradiographic studies after ^3H -TTP treatment.

Materials and Methods

Seeds of a variety "Svanhals" of barley (*Hordeum vulgare* var *distichon*) were germinated on filter paper in petri dishes. Twenty seeds dehusked over the embryo region for the partial synchronization of initial germination were soaked in 2 ml of distilled water (pH 5.5) at 25°C in the dark. The first leaves (14~16 pieces) were cut from germinating seeds every 2 hours during 0 to 24 hours after seed soaking, and immersed in 2 ml of a solution which contained ^3H -thymidine (thymidine-6- ^3H , specific activity 20.2 Ci/mM, The Radiochemical Centre, England) at a concentration of 2.0 $\mu\text{Ci/ml}$ or ^3H -thymidine triphosphate (thymidine methyl- ^3H -5'-triphosphate ammonium salt, specific activity 11.6 Ci/mM, The Radiochemical Centre, England) at 4.0 $\mu\text{Ci/ml}$. After treating in

the radioactive solution for 2 hours, the leaves were washed thoroughly with water and fixed in ethanol-acetic acid (3:1). The materials were hydrolysed in 1N HCl at 60°C for 17 minutes, and stained with the Feulgen reagent. Intercalary meristems of the first leaf were squashed in 45% acetic acid. The dry ice technique was used for removal of cover glasses. Slides were dipped in a vessel containing 50% of Sakura emulsion NR-M2. After exposure for 20 days in the cool shade, the slides were developed for 4 minutes in Konidol X. For mitotic index determination, 2,000 to 3,000 cells of intercalary meristems were scored for each of 8 slides prepared at every fixation times. For leaf meristems treated with ^3H -thymidine (^3H -TdR) or ^3H -thymidine triphosphate (^3H -TTP), a total of 1,000 to 1,500 cells was scored from 6 slides, and the percent of labelled cells to total scored cells and the proportion of prophase, metaphase and ana-telophase, which were labelled, were determined.

Results and Discussion

Fig. 1 showed the ^3H -thymidine triphosphate incorporation into the meristematic cells of barley. ^3H -TTP incorporated into the nuclei of meristematic cells as ^3H -TdR did. Labelling index with ^3H -TdR or ^3H -TTP treatment and mitotic index at various times after seed soaking was begun are shown in Fig. 2. With the incorporation of ^3H -TdR or ^3H -TTP, labelled nuclei appeared in the intercalary meristems after about 12-14 hours

(a)

(b)

Fig. 1 Autoradiographs of meristematic cells treated with tritiated thymidine triphosphate (a) and tritiated thymidine (b).

Fig. 2 Mitotic index, ●, and incorporation of ³H-thymidine, •, or ³H-thymidine triphosphate, ○, for 2 hours into the meristematic cells of the first leaf after seed soaking of barley at 25°C.

(Fig. 2; 13 hours) and the frequency increased rapidly to reach to a maximum of about 26% at 16-18 hour (Fig. 2; 17 hours)

or of 36% at 18-20 hour (Fig. 2; 19 hours), respectively, being almost the same increasing pattern of labelling index between both the treatments. Thereafter, the frequency of ³H-TdR labelled cells decreased rapidly to 2.0% at 22-24 hour (Fig. 2; 23 hours). ³H-TTP labelled cells also fell to about 18% at 20-22 hour (Fig. 2; 21 hours). At 20-24 hour after the initiation of seed soaking, percentage of ³H-TTP labelled cells was not so decreased as the frequency of cells labelled with ³H-TdR treatment (Fig. 2).

When germinating seeds were treated with tritiated TTP for 2 hours at 18 or 20 hour after soaking, the mitotic cells having silver grains on their chromosomes were found in the meristems as demonstrated in Fig. 3. Mitotic index of meristematic cells at 18 hour after the initiation of seed soaking was about 5% and increased to 8% at 20 hour. Mitotic cells were composed of prophase (55%), metaphase (14%), anaphase (10%) and telophase (21%), at 20-22 hour after seed soaking. About 47 to 64% of the nuclei in each phase mentioned above were labelled with more than 4-18 silver grains on the chromosomes. We have already estimated from the pulse and chase experiment with ³H-TdR that the duration of mitosis (M), premitotic phase (G₂), and DNA synthetic phase (S) was 2 hours, 4 hours and 4.5 hours respectively²¹. Therefore, labelled anaphase and telophase cells would have been at late G₂ and mitotic prophase when the leaves were immersed in ³H-TTP solution. Our experiment shows, thus, that a certain amount of DNA is presumably synthe-

Fig. 3 Mitotic cell having silver grains on the chromosomes.

sized during G₂ and mitotic prophase in the meristematic cells of a barley first leaf.

Hotta, et al.³⁾ reported using biochemical methods that only a small amount of DNA was synthesized during meiotic prophase in lily microsporocytes. Recently, cytological evidence of the DNA synthesis during mitotic prophase in cultured human leucocytes has been presented by Mukherjee, et al.⁴⁾ We were able to observe a similar DNA synthesis in the meristematic cells of plant when tritiated TTP was incorporated. When the first leaves were treated in tritiated TdR solution at 20 to 22 hour after soaking, however, no labelled mitotic cells was detected. The results obtained in this experiment seem to suggest that, if TTP is available, DNA synthesis can occur during G₂ phase and also support our inference that DNA polymerase is active in the post DNA synthetic cells⁵⁾.

References

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