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MASTER

AN ANALYSIS OF H^3 -THYMIDINE DISTRIBUTION THROUGHOUT THE SHOOT
APICAL MERISTEM OF ARABIDOPSIS THALIANA (L.) HEYNH¹

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Running title:

H^3 -Thymidine Distribution in the Shoot Meristem

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Numerous studies have been made on the kinetics and distribution of DNA synthesizing cells of the root meristem using the technique of autoradiography following immersion of the roots in tritiated thymidine. However, application of this technique to the shoot meristem has been very limited.

Gifford (1) stated that attempts to bring about incorporation of H^3 -thymidine into the shoot systems of intact seed plants have not always been successful, nevertheless with Chenopodium album a daily application over a period of three days of 10 $\mu\text{C}/\text{ml}$ of H^3 -thymidine preceded by application of 0.1% "Tween 20" as a wetting agent enabled him to demonstrate some labelled nuclei in the apical meristem following autoradiography of the processed sections.

In a previous paper, Partanen and Gifford (2) studied the distribution of DNA synthesis in the apical meristem of Pinus lambertina using P^{32} as the labelling substance, incorporated via the roots. Following a three day period of uptake the apical shoot was fixed, sectioned, extracted (3) and hydrolyzed. The residual label was considered to represent mainly P^{32} in DNA with some protein label in addition.

Though the autoradiographed sections showed a relatively uniform distribution of activity over meristem sections, Partanen and Gifford (2) suggested that the presence of highly radioactive centres in the "terminal apical zone" was evidence of DNA synthesis preceding cell division in this region. This evidence was considered to be opposed to the concepts of Buvat (4) and others: for review see Cutter (5), which holds that cells in the sub-apical region constitute a "meristem d'attente" which shows little division activity and consequently has no organogenic role during

vegetative growth.

Clowes (6, 7) studied the distribution of DNA synthetic activity in the shoot meristem of Coleus blumei using C^{14} adenine as the labelled precursor. This was injected (0.02 ml, activity 2 μ c, 0.08 mgm) into the stem just below the apex and non DNA specific label was presumed to be removed by N HCl hydrolysis. Autoradiographed sections of apices treated with adenine C^{14} for eight days before processing showed dense radioactivity over the apex. Clowes concluded that this evidence refuted the concept of a "meristeme de'attente" or quiescent center in the shoot apex, although such a quiescent center has been demonstrated by Clowes in the root meristem (8), most graphically in Zea mays following H^3 -thymidine treatment for 72 hours.

In view of the division of opinion on the morphogenetic significance of the histological zones apparent in the vegetative apex, further experiments on the shoot apex are indicated using the high resolution and DNA specificity provided by H^3 -thymidine.

The purpose of this report is to demonstrate that a six hour application of H^3 -thymidine to the apex of Arabidopsis thaliana (L.) Heynh can label the DNA synthesizing regions throughout the apical meristem, and to show that the pattern of labelling corresponds to the cytohistological zonation first described for Arabidopsis by Vaughan (9). This labelling pattern reveals a block of sub-apical cells with significantly less thymidine incorporation.

MATERIALS AND METHODS

The material studied was the sectioned apical meristem of an Arabidopsis thaliana plant, fixed 16 days after sowing. Such plants were grown aseptically in test tubes on mineral agar, under controlled conditions of a 12 hour photoperiod and constant 25° C tube temperature. H³-thymidine, with specific activity of 5 µc/ml in a solution of 0.1% Tween 20 and 1/4 strength Hewitt's mineral solution, was applied as a 20 µl drop by syringe to the axillary region of treated plants. After six hours, plants were harvested, the rosette leaves removed and the apex fixed in vacuum with CRAF fixative. The material was embedded in paraffin by means of a tertiary butyl alcohol series. The embedded material was sectioned at 4 microns, and the ribbons were adhered to gelatin subbed slides, deparaffinized in xylene, and passed through an ethanol-water series to distilled H₂O. The slides were dipped in Kodak NTB emulsion at 40° C, dried in the dark room and stored in light tight boxes at 0° C for 17 days. Slides were then developed with Kodak D19 developer, fixed, dehydrated via an ethanol series, and the coverslip mounted with euparal. In the slide analyzed, sequential longitudinal sections of the apex were defined by the youngest leaf primordium at the commencement of the series and continued through to the overlying leaf primordia. The enclosing leaves were well labelled and the emulsion background was very low.

The successive sections were studied at a magnification of X 800 with a Wild M20 microscope equipped with phase contrast optics and the Wild Drawing Apparatus. Using this apparatus the section outlines were drawn and the position of each reduced silver grain plotted throughout the depth

of focus of the emulsion.

The drawings were then copied on transparent acetate by means of the Haloid-Xerox copying process. These copies were superimposed and oriented so that the lines bounding the middle apex coincided. A composite photograph was then made from this assemblage and, in addition, individual photographs were made from each drawing. Each section on the slide was photographed under phase contrast focussed slightly above the level of the section in order to show some grains. Plates of the drawings and photographs accompanying this report were reduced to equivalent magnification.

RESULTS

The individual sections show only a few cells of the meristem to be labelled, but most of this label occurs on the flanks of each section or in the area below that occupied by the tunica cells. Occasionally, labelled cells are found in the first tunica layer. The composite produced from the superimposition of each drawing, shown in (j) of Fig. 2 reveals a distinct sub-apical middle region of low labelling bounded by more heavily labelled flank zones. This region corresponds to the central initiation zone described by Vaughan (9), and also to the location of the "meristeme d'attente" proposed by Buvat (4). Vaughan illustrates the Arabidopsis thaliana apex at an early stage in transition to reproductive development with a more pronounced dome shape than that shown here, with four to five layers of flank meristem and a greater depth of file meristem, i.e. the cells below the central initiation zone.

Our studies of the transition stages under both continuous and 12

hour photoperiods confirm Vaughan's essential interpretation (Miksche and Brown, unpublished data) and place the 16-day-old meristem under discussion herein at the late vegetative stage of development. The most juvenile leaf primordium (5th leaf pair) is shown in (A) of Fig. 1, which is 90° from the overlying leaf of the 4th leaf pair illustrated in (G-H) of Fig. 2. The modal rosette leaf number is 12 under growth conditions of constant 25°C and 12 hour photoperiod. Floral initiation is observed in the apex at about 21 days and earliest anthesis at 40 days. These parameters are, however, sensitive to marked fluctuations from the regulated environment.

In order to obtain a quantitative estimate of the degree to which the distribution of label follows the pattern of histological zonation, the following procedure was employed. Tangents were drawn to the periphery of each section outline, the dimensions of tunica cells were measured from the original phase contrast photographs and the numbers of cells within the first tunica layer were counted. Each section was then divided into layers corresponding to first, second and third tunica layers. This procedure is necessarily somewhat idealized, particularly since the third tunica layer is poorly organized in some sections. Each layer was then divided off into "cell" intervals corresponding closely to average observed cell dimensions. This subdivision is illustrated for one section in Fig. 3.

Fig. 3

The silver grains falling in each of 15 cell intervals from left to right were counted in the first tunica layer, those in each of 12 intervals in the second tunica layer, and those in each of 9 cell intervals of the third tunica layer. Five, four and three, cell intervals in the first, second, and third layers, respectively, were allotted to segments corresponding to the left, median, and right segments of the apex, so that the cells per segment were of equal number. The grain count totals are shown in Table 1.

The expected grain counts, shown in parentheses, in each segment and layer were computed on the basis of these being proportional to the respective column and row totals in Table 1. A chi-square test with 4 d.f. for goodness of fit gave a value of 49.37. Hence there is evidence (P less than .001) that the distribution of grains in the right, left and median sections is not the same over the three tunica layers. The largest contribution to heterogeneity is made by the median segment in the second tunica layer. In fact, the value $(O-E)^2/E = 18.01$ exceeds the tabulated value of chi-squared (4 d.f., $P 0.01$) = 13.3, and clearly is a significant contribution.

DISCUSSION

This result indicates that it is possible to obtain incorporation of H^3 -thymidine into the apical meristem of Arabidopsis following local application of the isotope solution for a period much shorter than treatment times previously reported.

Such a result is not obtained in all meristems examined and there

is no doubt that penetration of the labelling solution to the apex presents difficulties not encountered in treatment of the root meristem. Differential labelling of the histological zones of the shoot apex is, however, more likely to be demonstrated in shorter treatment times than the three and eight day periods used by Gifford and Clowes, respectively. Despite this, examination of the photomicrograph illustrating the paper by Clowes (6), on adenine C^{14} incorporation in Coleus, reveals a sub-apical zone in which the silver grain intensity, though high, is visibly less than that of the adjacent flank regions. In Clowes' work, the very long treatment time would tend to obscure any difference in the rate of synthesis within the apex and, in addition, the greater energy of C^{14} β radiation compared to that of H^3 lowers the resolving power of the technique in localizing the sites of greatest incorporation. Further criticism may be made on the grounds that the radiation level produced by this long exposure in the tissue may itself modify the normal synthetic activity within the apex (10). The possibility also exists that not all the incorporated label represents synthesized DNA. This latter objection would certainly apply also to the use of P^{32} in studies on DNA synthesis within the apex.

Further studies using H^3 -thymidine on the distribution of DNA synthesis within the shoot meristem are necessary in order to obtain a clearer understanding of the significance of differential activity at different stages of ontogeny. That such differential activity may have morphogenetic significance is indicated by the morphogenetic response reported by Brown (11), following application of thymidine analogues to the vegetative meristem of Arabidopsis.

SUMMARY

Sequential autoradiographed sections through the shoot apical meristem of Arabidopsis thaliana treated for six hours with a solution of H^3 -thymidine in combination with 0.1% Tween 20 and dilute mineral solution reveal a differential pattern of labelling, indicative of differential rates of DNA synthesis. This pattern corresponds to the histological zonation of the apex and reveals a sub-apical block of cells in which the amount of incorporation is significantly lower than in the adjacent flank cells.

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TABLE 1

Distribution of Grain Counts in the Apical Meristem

Zones	Segments						Total Grains	Total Cells per segment
	Right		Median		Left			
	O	E	O	E	O	E		
1st Tunica layer	134	(134)	96	(71)	48	(73)	278	35
2nd Tunica layer	137	(127)	33	(68)	94	(69)	264	28
3rd Tunica layer	59	(69)	47	(37)	37	(37)	143	21
Totals	330		176		179		685	84

Fig. 1.--Phase contrast photomicrographs (x 260) of sequential 4 micron sections through the shoot meristem of a 16-day-old Arabidopsis thaliana plant. Silver grains are shown in the developed film emulsion covering the sections. (A) Final section through a leaf primordia of the 5th pair. (B-H) Sequential sections through the apical meristem.

FIGURE 1

Fig. 2.--Outline drawings (x 260) corresponding to the photographs of Fig.

1. Each silver grain through the depth of the emulsion of the autoradiographed sections has been plotted. (B-H) Individual sections. (J) Composite of (B-H) produced by superimposing transparent acetate copies of these drawings.

FIGURE 2