The Induction of Biplanar Growth in Fern Gametophytes in the Presence of RNA Base Analogues

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Received July 29, 1968.

Abstract. The RNA base analogues, 5-fluorouracil, 2-thiouracil, and 8-azaguanine, inhibit the growth of Dryopteris borreri, but do not prevent the transition from filamentous to biplanar growth. Transition, which occurs only when the filament has developed to 4 or 5 cells, may be considerably delayed, due to inhibition of filamentous growth, but it always occurs when the critical cell number of the filament is reached. Furthermore, the inhibitors show only a marginal differential effect on biplanar compared to filamentous growth when the growth rates are determined from kinetic studies. It is suggested that the selective effects previously reported may result from the experimental techniques used, coupled with the actual growth characteristics of the gametophyte.

The fern gametophyte is suitable material for the study of processes which control the orientation of mitosis. The gametophyte develops initially as a filamentous protonema and then as a 2-dimensional plate of cells. During the filamentous stage all the cell divisions occur parallel to each other, whilst the onset of biplanar growth is marked by a cell division at right angles to the previous plane of division. The transition to biplanar growth requires the presence of blue light, gametophytes grown in red light remaining filamentous (5). The developmental morphology can thus be controlled by the spectral quality of the light.

There are a number of reports that various analogues of RNA bases (1, 2, 3, 8, 11, 12) and amino acids (2, 3, 11), and various antibiotics (2, 6, 7, 9) selectively prevent the transition from filamentous to biplanar growth in a range of ferns. The nature of these inhibitors suggests that the induction of biplanar growth requires the synthesis of new species of RNA and protein. As a preliminary to a study of the involvement of RNA in transition, the effect of 3 analogues of RNA, 5-fluorouracil, 2-thiouracil, and 8-azaguanine, on the morphological development of the gametophyte has been re-examined. The results reported in this paper show that these analogues do not specifically inhibit the transition from 1 to 2-dimensional growth of Dryopteris borreri, and that they have only a marginal differential effect on 2-dimensional compared to filamentous growth.

Materials and Methods

Spores of Dryopteris borreri (Newn), which had been collected locally by Dr. A. F. Dyer of this department, were from the same plant as those used by Dyer and King (4) in an investigation on the effect of light and temperature on the morphology and cytology of gametophyte development. The spores were filtered through 3 layers of lens tissue and grown on the surface of 100 ml of medium in blackened crystallizing dishes covered with a glass lid. The medium contained 0.51 g MgSO4*7H2O, 0.12 g KNO3, 1.44 g Ca(NO3)2*4H2O, 0.25 g KH2PO4, 17 mg FeCl3*6H2O, and 30,000 units of Mycostatin (Squibb, New York) per litre. The temperature was maintained at 17.5°C. Growth was partially synchronized by adopting a standard germination procedure of 48 hr darkness. 24 hr of 5 ft-c of red light (2 Grolux fluorescent tubes with a red glass filter [Cunningham, Dickson and Walker, Edinburgh]), followed by a second 48 hr of darkness (4). At the end of the germination procedure the dishes were transferred to either 22 ft-c or 53 ft-c of blue light [6 40 w Philp's No. 33 fluorescent tubes and a blue glass filter (Cunningham, Dickson and Walker, Edinburgh) with or without a sheet of frosted glass], or to 48 ft-c of white light (two Philp's No. 33 fluorescent tubes). The inhibitors, 5-fluorouracil, 2-thiouracil, and 8-azaguanine were then added at the end of the germination procedure.

The 1 dimensional growth rate was determined by counting the number of cells in 2 samples of 50 filamentous gametophytes taken from each dish at daily intervals, and applying rectilinear regression analysis. Only cells of the filament, i.e. not the rhizoidal cells, were counted. The filamentous growth rate was not determined after 50% of the gametophytes were biplanar in order to prevent bias from the slower growing individuals which had not reached the transition stage. Transition was measured by determining the percentage of biplanar gametophytes in each sample. The scatter of the mean cell number was very large once biplanar growth had commenced, and in order to measure the 2-dimensional growth rate, germinating spores were transferred to mineral medium solidified with 2% agar and the number of non-rhizoidal cells in the same sample of gametophytes were counted at daily intervals.
Results

By the end of the germination period an unequal division to form the basal cell plus the first rhizoid has occurred in 50% of the spores. This first cell division is completed during the lag period which occurs after the cultures have been transferred to continuous light. It is followed by a division (equal) of the basal cell to form a 2-celled filament. A further unequal division of the basal cell to form the second rhizoid and an equal division of the apical cell occur concurrently, thus producing a gametophyte consisting of 3 filamentous cells plus 2 rhizoids from the basal cell. Under these experimental conditions the apical cell continues to divide to form a filament, such that the increase in filament cell number is proportional to time. When the filament is 4 or 5 cells long, a cell division, parallel to the long axis, occurs in the third cell from the apex: the transition to biplanar growth. The extent of filament development necessary before transition can occur is described by the mean cell number of the filament at the time when 50% of the gametophytes have undergone transition. The growth of the young gametophyte thus consists of 3 phases: an initial lag period, a period of linear increase in cell number with time, and transition to biplanar growth (fig 1A). Each of these parameters has been used to determine the effect on growth of the added inhibitors.

**Effect of 5-Fluorouracil and 2-Thiouracil.** When spores were grown under 53 ft-c of blue light, both 5-fluorouracil and 2-thiouracil increased the lag period and reduced the subsequent filamentous growth rate without affecting the linearity of growth (fig 1A). Neither inhibitor prevented the induction of biplanar growth. Increasing concentrations of both 5-fluorouracil and 2-thiouracil affected the lag period and the cell division rate (growth rate), but the mean cell number at which transition occurred was unchanged (fig 2). Due to the effect on the lag period and on the division rate, the time of transition was later.

After transition, the biplanar growth rate was approximately twice that of filamentous growth (fig 3). The addition of 5-fluorouracil (5 µg/ml) and 2-thiouracil (2 µg/ml) both inhibited the 1 dimensional growth rate by 45%, while inhibiting the 2 dimensional growth rate by 65% and 55% respectively. There is therefore only a slight difference in the inhibition of 1 and 2 dimensional growth.

**FIG. 2.** The effect of inhibitor concentration on A) the lag period; B) the filamentous growth rate; C) the mean cell number at which 50% of the gametophytes are biplanar. Gametophytes were grown under 53 ft-c of blue light in the presence of 5-fluorouracil (■) and 2-thiouracil (○).

**FIG. 3.** Filamentous and biplanar growth rates of Dryopteris borreri. Gametophytes were grown under 53 ft-c of blue light on agar medium in the absence of (●) and presence of 5 µg/ml of 5-fluorouracil (■) and 2 µg/ml of 2-thiouracil (○). The time of 50% transition is marked by an arrow.

The inhibition kinetics of 5-fluorouracil and 2-thiouracil were different when the light intensity was reduced to a level which limited the growth rate. At 22 ft-c of blue light, in which the control filamentous growth rate was 12% slower than that at 53 ft-c, the increase in cell number in the presence of inhibitors was no longer linear with time (fig 1B), but transition still occurred once the filament had reached 5 cells.
Table 1. A Comparison of Inhibition in Blue (53 ft-c) and White (48 ft-c) Light

<table>
<thead>
<tr>
<th>% Inhibition of filamentous growth</th>
<th>Sel no. at 50 % transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>Blue</td>
</tr>
<tr>
<td>5-Fluorouracil (2 µg/ml)</td>
<td>39</td>
</tr>
<tr>
<td>2-Thiouracil (2 µg/ml)</td>
<td>51</td>
</tr>
</tbody>
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Very similar inhibitions were observed when white light (48 ft-c) was used instead of the higher blue light (53 ft-c), (table I).

Effect of 8-Azaguanine. Under 53 ft-c of blue light, 8-azaguanine increased the lag period and inhibited the subsequent filamentous growth rate. The inhibition was not, however, linear with time, since after a period cell division stopped (fig 4A). The cell number at which this occurred was dependent upon the concentration of the 8-azaguanine. At the lower light intensity (22 ft-c of blue light) there was a change in the pattern of the inhibition kinetics (fig 4B). The extension of the lag period was eliminated and there was a reduction in the initial inhibition of the growth rate. This difference between high and low light appears to be principally due to the longer lag period and slower growth rate of the control under the low light. Under both light intensities transition occurred provided that the filament reached a sufficient number (5) of cells.

![Fig. 4](image)

Fig. 4. The mean cell number per filament and the percentage of bplanar gametophytes during growth of Dryopteris borrer i under 53 ft-c (A) and 22 ft-c (B) of blue light. Gametophytes were grown in the absence (●) and presence of 0.1 µg/ml ([square]) and 0.2 µg/ml ([circle]) of 8-azaguanine.

Discussion

Under the experimental conditions used for the growth of Dryopteris borrer i, transition occurs when the filament reaches the 4 or 5 cell stage. The presence of 5-fluorouracil, 2-thiouracil or 8-azaguanine clearly does not prevent this transition to bplanar growth provided that the filament has reached this critical cell number (fig 2). The occurrence of transition in the presence of RNA base analogues contrasts with published results which claim that bplanar growth is selectively inhibited under these conditions (1, 2, 3, 8, 11, 12). The reported selective inhibition of transition by RNA base analogues does not appear to be specific, since it has been reported in Dryopteris borrer i (1) as well as in Asplenium nidus (8). Dryopteris erythrosora (3), Phymatodes nigrescens (12) and Pteridium aquinum (2, 11). Further, in these studies with Dryopteris borrer i, although the kinetics of inhibition varied with the different cultural conditions, such as the quality and quantity of the light, transition always occurred if the filament reached the required 4 or 5 cells, suggesting that the reported selective inhibition of transition is not peculiar to particular growth conditions. In actual fact, the observations presented in this paper are similar to those published by other workers (2, 8, 12), namely that gametophytes grown in the presence of an inhibitor are, at a fixed time, still filamentous whilst the control is 2 dimensional. (in fig 1 or fig 4 consider the control and inhibited gametophytes at 180 hr). However, in the presence of inhibitors, transition occurs at a later time when the filament has reached the 4 or 5 cell stage. This requirement for a certain amount of filament growth before transition is difficult to evaluate comparatively, since in some species transition occurs in the apical cell [e.g. Pteridium aquinum (2) and Phymatodes nigrescens (12)] whereas in others, such as Dryopteris borreri, it occurs in the third cell from the apex, which in itself necessitates a certain development of the filament. However, in Phymatodes nigrescens the filament reaches 4 or 5 cells before transition (12) and in Asplenium nidus, whose pattern of transition was not described, there appears to be a requirement for 6 or 7 filamentous cells before transition in the control (8). It is interesting to note that with these species, in the cases in which selective inhibition of transition was claimed, the cell number never exceeded, and seldom reached, this value (8, 12). Certainly in the case of Dryopteris borrer i, Pteridium aquinum (2) and Dryopteris filix-mas (10), the presence of inhibitors, such as 5-fluorouracil, 2-thiouracil, and 8-azaguanine, delays the transition from 1 to 2 dimensional growth, due to the general inhibition of the filamentous growth, but does not selectively prevent transition.

The inhibition of 2 dimensional growth rate by the analogues is only slightly greater than the inhibition of the filamentous growth rate (fig 3), and this could be due to the overall faster growth rate after transition. However, if measurements are made at a fixed time, then the inhibition of 1 dimensional growth, coupled with the filament development necessary for transition and the subsequent faster growth rate, can result in an apparent selective inhibition of 2 dimensional growth (8). Apparent selective inhibition is also observed if the 1 dimensional
growth (number of cells per filament) and the percentage of 2-dimensional gametophytes are determined at a single time (2). A small decrease in the filamentous growth rate results in a larger decrease in the percentage of biplanar gametophytes. (in fig 1, a 10% decrease in the growth rate reduces the percentage of biplanar gametophytes by more than 20%). This effect is further exaggerated if measurements are made on a population containing a high percentage of biplanar gametophytes, for in this case the 1-dimensional growth, which is determined from those gametophytes still present as filaments, is heavily biased towards the slower growing individuals. This low value of the filamentous growth under control conditions consequently minimizes the difference between the control and the inhibited cultures, which contain a smaller percentage of biplanar gametophytes and therefore do not give such a biased result. Thus assessment of the growth of the fern gametophyte at a single time by determining the number of cells per filament and the percentage of 2-dimensional gametophytes (2) tends to minimize the inhibition of 1-dimensional growth while magnifying the effect on 2-dimensional growth. Such considerations must also apply to studies on the effect of antibiotics on the induction of biplanar growth (2, 6, 7, 9).

The kinetic studies of both 1 and 2-dimensional growth rates presented in this paper show no selective effect by RNA base analogues on the development of the fern gametophyte.

NOTE ADDED IN PROOF

Since the submission of this paper, J. H. Miller (1968, Physiol. Plantarum 21: 699-710) has published results which similarly show non-selective inhibition of growth of fern gametophytes by 8-azaguanine.

Acknowledgments

We thank Dr. A. F. Dyer for the gift of the spores and for helpful discussions concerning the development of Dryopteris borreri, and Professor R. Brown for his interest and encouragement.

Literature Cited