

Fern Gametophytes as a Tool for the Study of Morphogenesis

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The purpose of this paper is to review those features of fern gametophyte morphology which render them uniquely valuable as subjects for the experimental morphologist; and to present the results of a preliminary study on the effects of biotin and α -naphthaleneacetic acid (NAA) on vegetative development of *Pteridium aquilinum* gametophytes.

Although not all of the following characteristics are unique to fern gametophytes, the aggregate effect of these characteristics contained within a single type of organism provides a subject of exceptional potential for investigating and analyzing certain basic problems in morphogenesis.

The first point of consideration is that of size. Fern prothalli, which usually range from about $\frac{1}{2}$ to 1 cm. in diameter at maturity, are small enough to be cultured in quantity in a small container such as a petri dish, yet large enough to permit surgical treatment under a binocular microscope, using simple tools. Albaum (2) has taken advantage of this feature to study regeneration in fern prothalli.

Another characteristic is that of the relatively short maturation period of two to six weeks in most species. This allows for fairly rapid evaluation of treatment effects and permits the execution of cumulative sequence experiments in a much shorter period than comparable studies on other photosynthetic land plants.

The next point is concerned with the vegetative development of fern prothalli. In most species of higher ferns, spore germination gives rise to a relatively brief filamentous stage. This is followed by a rather prolonged period of biplanar cell division, during which the prothallus assumes a cordate form as a consequence of the establishment of an apical initial cell. Finally, cell division in the apical region of the prothallus becomes three-dimensional, giving rise to a cushion of cells. Because the prothallus is only one cell layer in thickness during the biplanar stage of division, the following advantages obtain:

(1) Growth can be measured as a function of increase in surface area. As Albaum (1, 2) has shown, this enables the investigator to make a rapid assay of relative development, and to establish precise growth curves for individuals, rather than resorting to approximations from population samplings.

(2) Cell lineages, hence morphological development, can be followed throughout the organism. Dopp's (3) work in tracing the cell-for-cell development of a single prothallus points the way to a direct and comprehensive means for the investigation of growth at the cellular level.

(3) The sequence of effects of chemical or surgical treatments can be observed throughout the entire living organism, without resorting to the conventional method of killing the subject in order to observe it.

In addition to the foregoing advantages resulting from discrete stages in cellular growth patterns, fern prothalli provide a means for

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investigating morphogenesis at the biochemical level. Hotta, Osawa, and Sakaki (4, 5) have investigated the factors underlying the transition from filamentous to two-dimensional growth, and have found it to be reversible with certain amino acid analogs. They further discovered that the onset of the two-dimensional stage is accompanied by the production of a different type of RNA from that of the filamentous stage. This work may eventually provide an important bridge in the gap between what is known of biochemical reactions and what is known of the expression of those reactions at the morphological level.

The final feature deals with the patterns of prothallial development in relation to sex expression. These patterns have been elucidated by Naf (6, 7) who has found them to be related to the elaboration of a morphogen which he terms the antheridial factor. Because the appearance of this factor and its functions are integral to the developmental morphology of the prothalli, its characteristics will be briefly described.

First, it is a discrete biochemical entity, produced by the prothalli of polypodiaceous ferns and subsequently released into the culture medium. It has been demonstrated to be specific for the induction of antheridia, hence the name antheridial factor. The mode of action in the induction process has not yet been clarified, but it appears to function in a system of two, three or more competitive factors. Thus, the term antheridial factor may be more comprehensive than its present application warrants; nevertheless, it will be used in this discussion without further qualification.

Naf (6) has determined the time-course of the production of the antheridial factor which appears as follows in relation to the behavior of prothalli of *Pteridium aquilinum*.

Spores begin germination the second day after inoculation, and continue germinating through the first week. On the seventh day the first heart shaped prothalli are apparent, and on the eighth day the first effective concentration of antheridial factor can be detected; on the tenth day the first antheridia are initiated.

The development of archegonia does not occur until after the cushion of cells develops just behind the apical notch. The initiation of archegonia marks the end of susceptibility to the antheridial factor. That is, once archegonia have arisen on a prothallus, no antheridia can be induced on that prothallus.

The gametophytes in a culture fall into three general categories. The most rapidly growing prothalli produce archegonia, beginning at about the seventh day after inoculation. These gametophytes, "female" prothalli, apparently produce the antheridial factor, which diffuses into the medium and exerts its influence on the less rapidly growing prothalli. Because they have reached the stage of archegonial initiation before effective levels of antheridial factor have been produced, these "female" prothalli are insensitive to the factor and so remain exclusively archegoniate.

The second growth pattern is associated with what will be called "hermaphroditic" prothalli. These, being slower growing than the first group, have not reached the insensitive stage and so produce antheridia in response to the presence of the antheridial factor secreted by the "female" prothalli. They too produce the antheridial factor. However,

presumably because they have developed an apical initial region, they develop into cordate gametophytes, eventually giving rise to an archeogonial cushion and losing sensitivity to the factor. The antheridia thereon disintegrate by the time four archeogonia are produced. In a mature colony these are usually recognizable only as archeogoniate prothalli.

The third growth pattern, giving rise to exclusively antheridium-bearing prothalli, is characteristic of the slowest growing individuals in the culture. These prothalli have not formed an apical meristem at the time the antheridial factor becomes effective. Presumably this precocious production of antheridia retards prothallial growth to such an extent that these gametophytes remain depauperate and perennially antheridiate.

Thus, the previously mentioned work of Hotta and his co-workers opens the way to investigation of morphogenesis at one of its simplest levels of expression, i.e. cell division in a one-dimensional vs. a two-dimensional plane. And Naf's work provides the basis for morphogenic investigation at the much more complex level of sex expression and the attendant growth-differentiation interactions.

A preliminary investigation on response of fern prothalli to growth regulators was undertaken by the authors. Biotin was used because of its function in rapidly growing tissues in carboxylation and decarboxylation in the TCA cycle, and in deamination of amino acids, presumably operating in protein synthesis. NAA was selected on the basis of its auxin activity.

Spores of *Pteridium aquilinum* were seeded on nutrient agar (1%) containing inorganic salts as follows:

Moore's solution

NH_4NO_3	500 mg/1
KH_2PO_4	200 mg/1
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	200 mg/1
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	100 mg/1

Nitsch's solution (modified) at 1 ml/1 culture medium

$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	3.000 g/1
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	500 mg/1
H_3BO_3	500 mg/1
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	25 mg/1
$\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$	25 mg/1
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	25 mg/1
H_2SO_4 (sp. gr. 1.83)	0.5 ml/1

Biotin and NAA were added after the medium was autoclaved but before it solidified, to give the following concentrations: NAA, 10^{-9}M through 10^{-8}M ; Biotin 10^{-6}M through 10^{-3}M ; and Biotin at 10^{-6}M or 10^{-8}M in combination with NAA at 10^{-9}M or 10^{-8}M .

The cultures were grown in pyrex petri dishes at 23°C , under ca. 200 f.c. of continuous light (G.E. Standard Cool White fluorescent).

Notes on development were recorded at different intervals for a period of approximately six weeks. At the end of this time, measurements of maximum length and maximum width of prothalli were made, using an ocular micrometer on a dissecting microscope. Some of the materials were judged unsuitable for the collection of size data, owing to variations

attributable to factors in the cultural environment. The measurements were converted to metric equivalents, and the product of maximum length X maximum width was used as an index of surface area, hence growth [after Albaum (1)]. The results are shown in Table 1.

Table 1. Effect of growth regulators on size of *Pteridium aquilinum* gametophytes (6 weeks)

TREATMENT	AVERAGE RELATIVE SIZE (length X width in mm.)	
	"Male" Prothalli	Other Prothalli
CONTROL	1.2	35.0
BIOTIN 10^{-6} M	1.4	31.1
10^{-4} M	1.6	40.0
10^{-3} M	2.2	37.9
NAA 10^{-6} M	1.3	29.1
10^{-7} M	1.2	15.6
10^{-8} M	0.2	1.4
BIOTIN 10^{-6} M	1.6	37.6
NAA 10^{-6} M		
BIOTIN 10^{-6} M	1.2	39.6
NAA 10^{-6} M		
BIOTIN 10^{-5} M	5.7	39.6
NAA 10^{-6} M		

These data reveal three features of possible significance. First, there appears to be a genuine, albeit small, effect of increased growth due to the addition of biotin, especially upon the "male" prothalli. Observations indicate that this stimulation was effected most strongly in the early stages of growth, in which there was a pronounced size increase in the biotin treatments over the controls.

Conversely, there was decided inhibition of growth by NAA at the higher concentrations. This corresponds with the findings of Soussentov (8). Although several of the NAA-treated prothalli in the present study produced antheridia, no archegonia were produced on the prothalli in the 10^{-6} M concentration of NAA. This effect is considered to be indirect, as there was no formation of the cushion of cells which seems to be a prerequisite to archegonial initiation.

Finally, the combined application of biotin at 10^{-5} M and NAA at 10^{-6} M had a pronounced effect on growth of the "male" prothalli. The growth promoting effect of this combination was readily apparent in all treated prothalli from early stages of development. The fact that the measurements made at the end of six weeks' growth do not strongly corroborate these observations suggests that there may be a size limiting factor which tends to equalize mature size of both stimulated and control prothalli. If this is so, the measurements may have been made too late to demonstrate the full effectiveness of the biotin. This suggestion is further implemented by the following observations on the form of the "male"

prothalli. Even in the controls a few of these usually ameristic prothalli produce three, four, or more separate apices, resulting in a rosette of cordate appendages, each of which looks like a typical heart-shaped prothallus in miniature. The frequency of such rosette prothalli was greater in the biotin-treated cultures, and still greater with the biotin-NAA combination. In addition, the latter treatment appeared to stimulate vegetative growth to the extent that one or more of the apices on some prothalli would develop into almost normal-sized cordate prothalli.

From this it is apparent that biotin and, to a greater extent, the appropriate proportions of biotin and NAA stimulate the rate and degree of development of active meristems. It would seem, therefore, that the chief influence is upon the rate of cell division. It may be that there is an additional effect of promoting polar orientation and consequent inception of an apical region of meristematic cells.

Summary

The potential value of fern gametophytes as a subject for studies of morphogenesis lies in their suitability of size; short period of maturation; vegetative growth sequence from filamentous to biplanar to three-dimensional cell division; and patterns of prothallial development in relation to sex expression.

Preliminary studies on the effect of biotin and NAA on prothallial development indicate that NAA at 10^{-6} M inhibits cell division, and has the indirect effect of preventing archegonial initiation. Biotin appears to progressively stimulate growth through the concentration range of 10^{-6} to 10^{-3} M, and the combination of biotin and NAA, particularly at 10^{-5} M and 10^{-9} M, respectively, further promotes growth. It is suggested that this stimulatory effect is primarily upon the rate of cell division.

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