

## Development of antheridial filaments of *Chara vulgaris* L. in isolated antheridia *in vitro*

HANNA KURAN, KAZIMIERZ MARCINIAK

Department of Plant Cytology and Cytochemistry, Institute of Physiology and  
Cytology, University of Łódź, Banacha 12/16, 90-237 Łódź, Poland

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### Abstract

The study was undertaken to establish the conditions of culture *in vitro* of *Chara vulgaris* L. antheridium isolated from the mother plant and to ascertain the regularities in the development of the antheridial filaments under these conditions. The culture of isolated antheridia was run for 5 days on Forsberg medium. The antheridial filaments were found to preserve their full viability evaluated in terms of  $^3\text{H}$ -phenylalanine incorporation with simultaneous depression of mitotic activity. Under these conditions a considerable part of the filaments pass through at least three division cycles. *In vitro* culture of isolated antheridia causes the greater shortening of the cell length the longer the antheridia are kept isolated from the thallus.

### INTRODUCTION

The antheridial filaments of *Chara vulgaris* develop within antheridia forming on the pleuridia arranged in whorls. Owing to naturally synchronised mitotic divisions there develop from one cell successively 2-, 4-, 8-, 16-, 32- and 64-cell antheridial filaments. After each successive division the sizes of the cells become reduced by  $1/3$  as compared with those of the preceding generation. DNA synthesis starts as early as telophase and is continued only in the early period of interphase. The cycle is of  $\text{S}+\text{G}_2+\text{M}$  type (Olszewska and Godlewski 1972).

With the limitation of cell growth there gradually occurs a limitation of RNA and protein synthesis (Olszewska and Godlewski 1972). The decrease of the cells length and the reduced incorporation of labelled RNA and proteins precursors is connected with the progressive

shortening of the duration of phase G<sub>2</sub> (Godlewski and Olszewska 1973), thus also of the whole cell cycle.

The aim of the present study was to establish the conditions of *in vitro* culture of isolated *Chara vulgaris* antheridia and the regularities in the development of the antheridial filaments under these conditions so that they could serve as material for investigations on the regulation of the cell cycle and cell differentiation.

#### MATERIAL AND METHODS

The antheridia were isolated from *Chara vulgaris* plants taken from a natural environment. The antheridia cut off from the first and second whorl pleuridium were sterilised for 5 min in 0.01 per cent chlorhexidin diacetate and washed with sterile Forsberg medium (1965) with 0.75 per cent sucrose and 0.06 M mannitol added. The isolated antheridia were cultured in this medium. Introduction of sucrose into the incubation medium as a source of carbon and energy was based on the results of Landgren (1976) according to which cell divisions in pea cultured *in vitro* were not observed in a medium containing glucose, but only in the presence of sucrose. Mannitol in the incubation fluid served as osmotic protection of the cells (Landgren 1976). In Erlenmayer flasks of 50-ml capacity 100 antheridia were placed in each in 20 ml of sterile medium. The culture was aerated on a Unipan shaker at a 50 amplitude. The experiments were run for 5 days at 23°C, L:D = 14:10. For illumination of the culture a set of fluorescent tubes Flora was applied.

Antheridia for examination were taken at 24-h intervals for the following determinations:

- 1) of mitotic activity expressed by the mitotic index,
- 2) of the length of telophase cells,
- 3) of the degree of asynchronusly,
- 4) of initiation of new antheridial filaments,
- 5) of protein synthesis evaluated on the basis of <sup>3</sup>H-phenylalanine incorporation,
- 6) of DNA synthesis according to <sup>3</sup>H-thymidine incorporation.

The material was fixed in a mixture of absolute alcohol and glacial acetic acid (3:1) in the period of highest mitotic activity under conditions of natural photoperiod at 1 p.m. (Godlewski and Maciejewska 1972). Squashes were stained with an orcein and Fast Green mixture. In the experiment with phenylalanine (2 µCi/ml, spec. act. 77 Ci/mM) the material was incubated for 2 h, the preparations were covered with liquid Ilford K2 emulsion and exposed for 20 days. In the

experiment with thymidine (30  $\mu\text{Ci/ml}$ , spec. act. 21.6 Ci/mM) incubation lasted 6 h and exposure 8 months. The autoradiograms were stained with Unna mixture. The control material consisted of antheridia of the first and second pleuridium whorls of plants from a natural environment.

## RESULTS

### LENGTH OF TELOPHASE CELLS

In 2-, 4-, 8-, 16- and 32-cell generations the telophase cells length is reduced with prolonged time of culture of the isolated antheridia in the medium (Fig. 1). In stages of 8, 16 and 32 cells the length of telophase cells is considerably shorter, beginning with the second 24 h of the experiment, as compared with the length of control antheridial filaments. The smallest size was reached after 96 h of culture. The cells of 2-cell telophase filaments are least reduced.

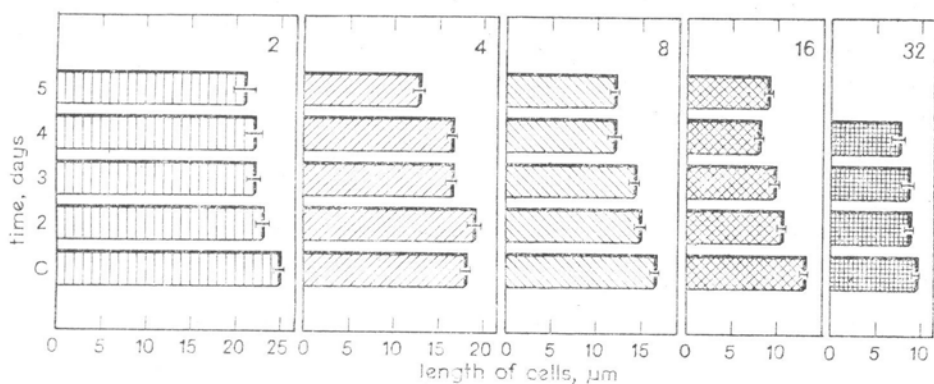


Fig. 1. Length of telophase cells in antheridial filaments in 2-, 4-, 8-, 16- and 32-celled generations. C — control, 2, 3, 4, 5 — successive days of *in vitro* culture of isolated antheridia

### MITOTIC ACTIVITY

During the first 24 hrs after isolation of the antheridium an abrupt depression of mitotic activity was observed, with the exception of the 32-celled antheridial filaments. Beginning with the second 24 h after isolation of the antheridium the antheridial filaments show a high mitotic activity (Table 1), the 32-cell generation excepted in the 5th 24 h of the experiment. The existence of mitotic activity in 1-cell filaments in the second, third and fourth 24 h points to an initiation of new antheridial filaments in isolated antheridia.

Table 1

Mitotic activity in antheridial filaments from control and isolated antheridia

Stage of development of antheridial filaments	Antheridial filaments in control antheridia, %	Antheridial filaments in isolated antheridia, %				
		1st day	2nd day	3rd day	4th day	5th day
1-celled	12.8	0.0	4.6	8.4	1.5	0.0
2-celled	16.2	0.8	6.9	8.1	13.1	8.2
4-celled	22.9	0.6	11.3	30.3	8.2	18.8
8-celled	22.1	0.7	20.4	22.1	8.5	26.8
16-celled	23.8	1.3	50.6	15.8	8.4	15.0
32-celled	16.2	24.0	50.0	18.7	14.8	0.0

#### ASYNCHRONY IN THE DEVELOPMENT OF ANTHERIDIAL FILAMENTS

Asynchronous antheridial filaments appear in the control material among the 2-, 4-, 8-, 16- and 32-cell filaments, their percentage increasing with development (Table 2). The antheridial filaments developing in isolated antheridia show as a rule a lower degree of asynchrony than do those in the corresponding stages of controls.

Table 2

Asynchronously developing antheridial filaments in control and isolated antheridia

Stage of development of antheridial filaments	Asynchronously developing antheridial filaments in control antheridia, %	Asynchronously developing antheridial filaments in isolated antheridia, %				
		1st day	2nd day	3rd day	4th day	5th day
2-celled	0.9	0.0	0.0	0.0	0.0	0.0
4-celled	2.6	0.4	0.6	3.0	2.9	2.7
8-celled	7.0	0.0	8.0	15.3	3.6	12.0
16-celled	20.4	0.6	28.1	9.0	8.8	10.8
32-celled	32.9	12.4	15.9	26.7	23.9	2.3

#### PROTEIN SYNTHESIS

<sup>3</sup>H-phenylalanine incorporation into the cells of antheridial filaments in antheridia detached from the thallus remains at the same level as in the control filaments. In the successive development stages of the filaments, both in isolated and in control antheridia incorporation of

this precursor gradually diminishes (Fig. 2). The time of culture of the antheridia *in vitro* has no effect on the rate of  $^3\text{H}$ -phenylalanine incorporation.

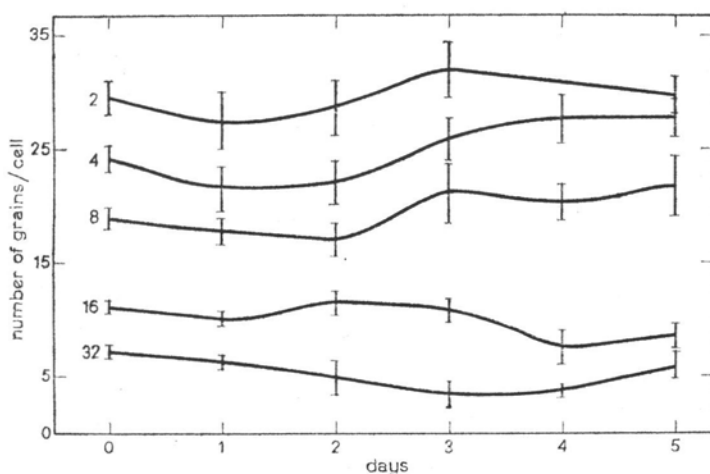


Fig. 2. Incorporation of  $^3\text{H}$ -phenylalanine into the cells of antheridial filaments of control plants (0) and of isolated antheridia after 1, 2, 3, 4 and 5 days of culture *in vitro*. 2-, 4-, 8-, 16- and 32-cell generations

#### DNA SYNTHESIS

The percentage of nuclei incorporating  $^3\text{H}$ -thymidine is lower in the antheridial filaments from isolated antheridia than in the controls (Table 3). The absence of isotope incorporation in 1-cell filaments in the second and third 24 h after cutting off the antheridium from the thallus is due to the initiation of new filaments at this time and the passage to the 2-cell stage of the already existing ones. In the first 24 h after isolation of the antheridium from the thallus the filaments of

Table 3

Cells incorporating  $^3\text{H}$ -thymidine in control and isolated antheridia

Stage of development of antheridial filaments	Antheridial filaments in control antheridia, %	Antheridial filaments in isolated antheridia, %		
		1st day	2nd day	3rd day
1-celled	30.0	6.2	0.0	0.0
2-celled	59.0	17.2	7.1	23.0
4-celled	61.9	14.2	9.8	49.1
8-celled	65.0	34.5	19.3	35.6
16-celled	89.0	16.6	23.0	44.5
32-celled	70.0	0.0	6.6	10.0

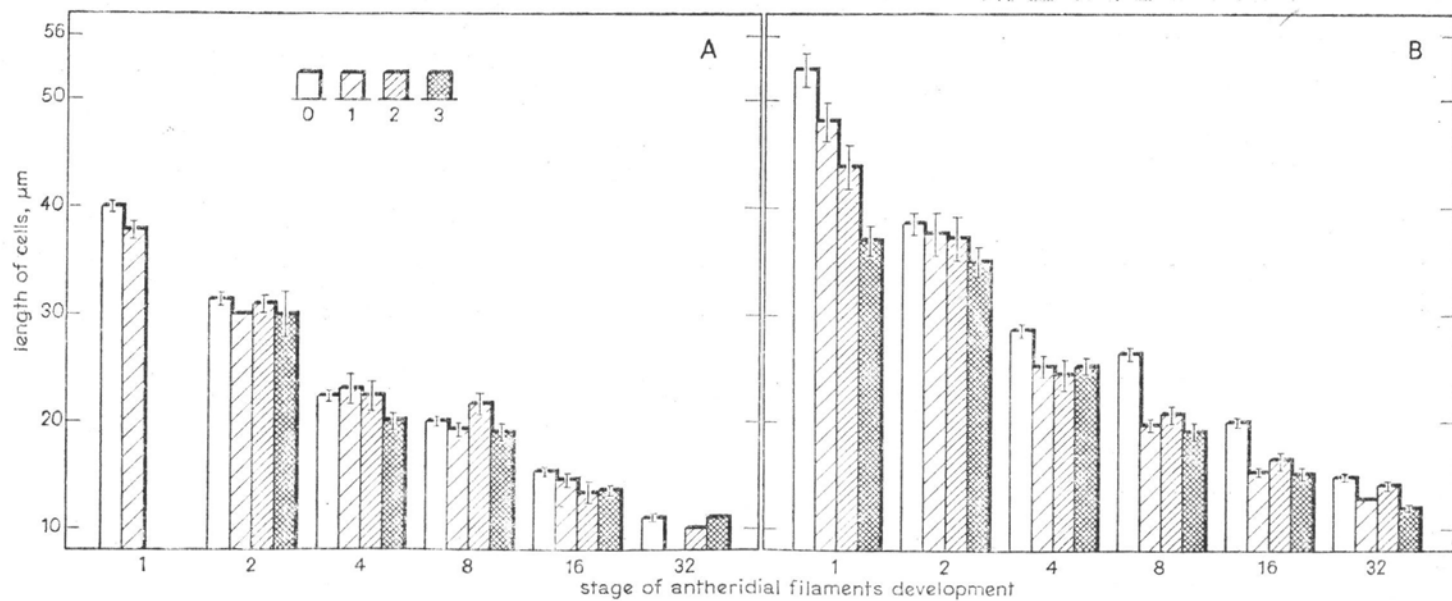


Fig. 3. Length of cells of antheridial filaments incorporating (A) and nonincorporating (B) <sup>3</sup>H-thymidine in control filaments and developing in isolated antheridia. O — control antheridia. 1, 2, 3 — isolated antheridia after 1, 2, 3 days of culture *in vitro*

the 32-cell generation do not incorporate  $^3\text{H}$ -thymidine; it is possible that they may have already been in phase  $G_2$  as indicated among other things by their high mitotic activity (Table 1). Evaluation of the length of  $^3\text{H}$ -thymidine-labelled cells as compared with that of cells in phase  $G_2$  (unlabelled nuclei) shows that in all the examined developmental stages of the filaments a considerable reduction of cell length occurred in this phase, particularly pronounced in 8-, 16- and 32-cell filaments (Fig. 3). This agrees with the results showing a reduction of cell length in telophase (Fig. 1).

#### DISCUSSION

$^3\text{H}$ -phenylalanine incorporation occurring with a similar intensity as in control material over 5 days of the experiment (Fig. 2) and  $^3\text{H}$ -thymidine incorporation indicate that *Chara vulgaris* antheridial filaments in isolated and *in vitro* cultured antheridia preserve their full viability. Labelling with  $^3\text{H}$ -thymidine and the mitotic activity under *in vitro* conditions indicate, however, a depressed ability of division. Nevertheless a large part of antheridial filaments passed in the experiment at least through three division cycles.

Telophase cells initiating the next cell cycle become somewhat shorter, the less the longer period of time the antheridia have remained isolated from the thallus. A confirmation of these results are measurements of cell length in the  $G_2$  phase (Fig. 3). A great reduction of the length of telophase cells (Fig. 1) and of cells not incorporating  $^3\text{H}$ -thymidine (Fig. 3) observed in more advanced (4-, 8-, 16- and 32-cell) stages of antheridial filaments development suggests that the older the cell generation the more pronounced is the reaction to isolation from the thallus. On the basis of the correlation between cell length in the successive generations and the duration of the cell cycle (Godlewski and Olszewska 1973, Olszewska 1974) it may be supposed that in antheridial filaments developing in isolated antheridia *in vitro* a shortening of the duration of the cell cycle occurs.

In isolated material the percentage of asynchronous filaments does not increase and in the 16- and 32-cell generation it is even lower than in antheridia developing on the thallus. Thus, the *in vitro* conditions do not cause desynchronisation of the cell cycle rhythm.

Cutting the antheridium from the thallus, severing the developing filaments from the nutrient and growth substances transported from the thallus causes a minimalisation of the conditions of development of the antheridial filaments, manifested in a certain inhibition of cell growth, a depression of the index of  $^3\text{H}$ -thymidine labelling and a lower mitotic activity in some filament generations.

Isolated antheridia cultured *in vitro* may, therefore, be a convenient material for studying the influence of exogenic factors on the course of the cell cycle and cell differentiation.

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#### Rozwój nici spermatogenicznych *Chara vulgaris* L. w izolowanych plemniach hodowanych *in vitro*

#### Streszczenie

Hodowlę izolowanych plemni prowadzono przez 5 dni w pożywce Forsberga z dodatkiem 0,75% sacharozy i 0,06 M mannitolu. Wykazano, że nici spermatogeniczne zachowują pełną żywotność ocenianą na podstawie włączania  $^3\text{H}$ -fenyloalaniny. Zauważono pewne obniżenie zdolności do podziału wyrażające się spadkiem aktywności mitotycznej, oraz zmniejszenie intensywności włączania  $^3\text{H}$ -tymidyny. Pomimo tego, znaczna część nici spermatogenicznych przechodzi co najmniej trzy cykle podziałowe. Hodowla *in vitro* izolowanych plemni powodowała skracanie komórek, tym znaczniejsze im dłużej trwała hodowla.