VELOCITY OF CYTOPLASM STREAMING IN BASAL AND SUBBASAL CELLS OF ANtheridium AS WELL AS INTERNODAL CELLS OF PLEURIDIum IN Chara Vulgaris L. AND GA₃ INFLUENCE ON IT. VIDEOMICROSCOPIC OBSERVATIONS

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ABSTRACT

The velocity of cytoplasm streaming in an antheridal basal cell and in a subbasal cell as well as in intermodal cells of pleuridia carrying antheridia were measured with the use of videomicroscopy. Velocity of streaming proved different depending on a cell type. The most intensive streaming (ca 40 μm/s) was observed in a subbasal cell while in a basal cell it was quite intensive during antheridal filament cells proliferation but falling to half of it during spermatozoid differentiation (ca 20 μm/s and 10 μm/s respectively). In intermodal cells of pleuridia the velocity was ca 17 μm/s.

GA₃ at the 10⁻⁶ M concentration decreased the velocity of streaming in a basal cell during proliferation of antheridal filament cells and increased it during spermigenesis. In intermodal cells of pleuridia the velocity diminished while in a subbasal cell it rose a little after GA₃ administering.

The obtained data suggest that cytoplasm streaming and its reaction to exogenous gibberellin depend on the role of a cell in a multicellularate system; it also depends on a developmental stage.

KEY WORDS: cytoplasm streaming, Chara vulgaris, basal and subbasal cells, intermodal cells.

INTRODUCTION

Various authors point out that intracellular streaming in Charales is generated by a actino-myosin system (Kamiya 1986; Kuroda 1990; Sievers et al. 1991; Nagai 1993; Staves 1997; Braun and Wasteneys 1998). Myosin isolated from Chara cells is structurally similar to muscle myosin V and is characterised by very high activity. In vitro it is able to move muscle actin fibres 10 times faster than myosin from muscles (Hagashi-Fujime et al. 1995).

The typical material for studying intracellular streaming in Charales were multinuclear cells of thallus main axis internodes convenient for observations due to their size (Staves 1997) as well as cells with apical growth: rhizoids and protonemata of Characean algae (Hejnowicz et al. 1985; Buchen et al. 1991; Sievers et al. 1991; Braun and Sievers 1994; Katembe et al. 1997; Braun and Wasteneys 1998; Braun and Richter 1999; Ackers et al. 2000). We thought it interesting to find out whether velocity of intracellular streaming depended on cell position and function, and developmental stage of an organ.

The data concern specific cells i.e. 1) an antheridal basal cell joining antheridium and thallus; 2) a subbasal cell in a pleuridium node which occupies a specific position and has plasmodesmal connections with antheridal and oogonial basal cells (Kwiatkowska and Maszewski 1986, unpubl. data); 3) intermodal cells of pleuridium coming from the first and second nodes of main axis of thallus Chara vulgaris (Fig. 1).

Subbasal and basal cells were examined during proliferative phase of antheridal filament cells, whose stages are marked by the number of cells in antheridal filaments, and during spermigenesis (differentiation of spermatozoid) which in Chara vulgaris starts most often at 64-cell stage. Spermigenesis is characterised by deep changes in nucleus and in spermaticid cytoplasm which made it possible to distinguish 10 spermigenesis phases in a light microscope (Popołńska 1999; Kwiatkowska and Popołńska 2002).

Since gibberellin plays an important role in Chara vulgaris morphogenesis (Godlewski and Kwiatkowska 1980; Kwiatkowska and Godlewski 1980, 1988; Kwiatkowska 1991, 1995; Kaźmierczak et al. 1999) its influence on ve-
locity of cytoplasm streaming was examined. GA₃ at the concentration of 10⁻⁵ M was used as this concentration showed significant influence on antheridial filaments development and thallus growth in previous studies.

MATERIALS AND METHODS

Apical parts of Chara vulgaris thallus from an experimental pond in Department of Cytophysiology, University of Łódź, Pilaarskiego 14 were the material. The plants were grown in tanks with water from the pond in the conditions of photoperiod similar to the natural (L:D=14:10).

Apical parts of Chara vulgaris thallus with antheridia at different developmental stages were incubated: a) for 1 week in 10⁻³ M GA₃ solution (water from the pond); b) for 1 week in water from the pond (control). After incubation, the material was placed in a microchamber made of plastic foil with pond water with or without GA₃ (the same solution as that in which they previously were grown). The microchamber was placed between a slide and cover slip. Cytoplasm streaming in the cells was observed in Optiphot-2 (Nikon) microscope and recorded on a monitor screen with Panasonic (CL702) camera. The velocity of movement was measured with a stop-watch during recording on a monitor screen. Subsequently filaments were squeezed out of the antheridium in order to examine the stage of development precisely. The distance covered by cellular structures was calculated on calibrated drawings made on a foil placed on the monitor. An average speed was calculated on the basis of the observation of ca six basal and subbasal cells for each spermiogenesis phase.

The results were statistically analysed with t-Student test with significance level p 0.05.

RESULTS AND DISCUSSION

The data show that intracellular streaming velocity depends on the type and function of a cell.

The fastest streaming was observed in a subbasal cell symplastically connected with two gametangia: male – antheridium and female – oogonium by their basal cells (Fig. 1). The velocity was about 40 µm/s and was roughly the same during all spermatogenesis phases (statistic difference - insignificant) (Fig. 2). The cytoplasm in an antheridal basal cell is less mobile than cytoplasm in a subbasal one and its streaming depends on a developmental phase of antheridium (statistic difference - significant) (Fig. 3). During proliferative phase of spermatogenesis it is ca 20 µm/s till a 16-cell stage. During spermiogenesis at all phases starting from phase I to the formation of mature spermatids – phase X – cytoplasm streaming is about 10 µm/s.

Experiments with GA₃ at the concentration 10⁻⁵ M showed that this hormone significantly changed cytoplasm streaming in a basal cell of antheridium at both spermatogenesis phases. During a proliferative phase it decreased while during spermiogenesis significantly increased streaming velocity (Fig. 3).

Streaming velocity in a subbasal cell after GA₃ administration rose to ca 50 µm/s in all spermatogenesis phases (Fig. 2).

![Fig. 2. Streaming velocity of antheridial subbasal cell cytoplasm during proliferation (1, 2, 4, 8, 16, 32) and spermiogenesis (I-X) (statistic difference-insignificant).](image)

![Fig. 3. Streaming velocity of antheridial basal cell cytoplasm during proliferation (1, 2, 4, 8, 16, 32) and spermiogenesis (I-X).](image)
In the light of our previous studies concerning GA₃ role in regulation of morphogenesis in Chama (Godlewski and Kwiatkowska 1980; Kwiatkowska and Godlewski 1980; Kazmierczak et al. 1999) the results seem to indicate that changing reaction of a basal cell to GA₃ was caused by a different character of streaming in both phases of spermatogenesis and/or by varied demand of antheridial cells for gibberellins depending on a developmental stage. During a proliferative phase the level of endogenous gibberellins was probably high in a basal cell which was manifested by its intensive metabolism. It elongated intensively and endomitotic DNA synthesis as well as active incorporation of radioactive precursors of RNA and proteins took place (Malinowski and Muszewski 1994). Capillary electrophoresis showed that a proliferative phase in antheridium development was characterised by a very high GA₃ level (Kazmierczak et al. 1999). Thus it may be suggested that GA₃ added to medium enhanced the level of gibberellins in a basal cell to a supraoptimal level which decreased metabolic activity and velocity of cytoplasm streaming. On the contrary when symptomatic connections between basal cell and antheridium were broken at 16-cell antheridial filament stage (Kwiatkowska 1988) most likely import of GAs from pleuridium decreased, as plasmodesmata were the main routes of GAs transport (Kwiatkowska 1991). The level of endogenous gibberellins in antheridium dropped significantly (Kazmierczak et al. 1999) probably leading, with some delay to their decrease in a basal cell. In this new physiological situation GA₃ added to medium restored a high level of gibberellins in a basal cell causing metabolic activity increase in that cell manifested by enhanced velocity streaming.

In order to check the above hypothesis we measured the velocity of cytoplasm streaming in intermodal cells of pleuridium which react, as it was previously observed, to exogenous GA₃ at the concentration 10⁻³ M with the decrease in metabolic activity expressed by limited elongation (Kwiatkowska and Godlewski 1980). Figure 4 shows that GA₃ at this concentration caused statistically significant decrease in cytoplasm streaming velocity in intermodal cells of pleuridium which supports our interpretation of the obtained data concerning basal cells.

Different character of streaming in a subbasal cell as compared to an antheridial basal cell may be due to its specific position between gametangia characterised with different developmental rate. For monoecious species such as Chama “protrandria” – earlier maturation of antheridium than of oogonium is characteristic (Gerlesquin 1959). Observations concerning growth dynamics of both gametangia showed that a rapid elongation and widening of oogonia started after antheridia reached a 16-cell stage of antheridial filaments i.e. after sympodial isolation of antheridium from thallus and then continued during spermiogenesis and after antheridium degradation, as well as after transformation of oogonium into oospores (Kwiatkowska et al. 1997). Thus during the whole period investigated by us a subbasal cell was a mediator between thallus and active acceptors of assimilates i.e. first antheridia and oogonia (Schulte et al. 1994) and then only oogonia which accumulated nutrients. Continuous intensive cytoplasmic streaming facilitating transport in a subbasal cell seems to be connected with its specific function.

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**PRĘDKOŚĆ RUCHU CYTOPLAZMY**

**W KOMÓRCE BAZALNEJ I SUBBAZALNEJ ANTERYDII**

JAK RÓWNEJW W KOMÓRKACH INTERNODALNYCH PLEUREYDIUM

U CHARA VULGARIS L. ORAZ WPŁYW GA₃ NA TEN PROCES

**STRESZCZENIE**

Prędkość przepływu cytoplazmy w komórce bazalnej anterydostanu oraz w komórce subbazałnej i komórkach internodalnych pleureydiów niosących anterydostany mierzono za pomocą videomikroskopii. Prędkość ruchu wewnątrzkomórkowego jest różna w zależności od typu komórki. Zaobserwowano najintensywniejszy ruch (około 40 μm/s) w komórce subbazałowej, zaś w komórce bazalnej anterydostanu jest on dość intensywny w fazie proliferacji komórek nici spermatogenicznych, ale o połowę niższy w okresie różnicowania plemników (odpowiednio ok. 20 μm/s i 10 μm/s). W komórkach internodalnych pleureydiów cytoplazma przemieszczała się z prędkością ok. 17 μm/s.

GA₃ w stężeniu 10⁻⁵ M powoduje obniżenie prędkości ruchu w komórce bazalnej w okresie fazy proliferacyjnej nici spermatogenicznych i jej zwiększenie w okresie spermiogenezy. Po działaniu GA₃, obniżeniemu ulega także prędkość ruchu w komórkach internodalnych pleureydiów, natomiast w komórce subbazałnej zwiększa się w niewielkim stopniu.

Na podstawie uzyskanych danych można przypuszczać, że ruch cytoplazmy i jego reakcja na egzogenną giberelinę zależy od roli komórki w wielokomórkowym systemie; zależy on także od stadium rozwojowego organu.

**SŁOWA KLUCZOWE:** ruch cytoplazmy, Chara vulgaris, komórka bazalna i subbazałna, komórki internodalne.