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

ŚLAWOMIR MALINOWSKI, MARIA KWIATKOWSKA, KATARZYNA POPŁOŃSKA,
ŚLAWOMIRA KNAKE, ANNA SOBALA

Department of Cytophysiology, University of Łódź
Piłarskiego 14, 90-231 Łódź, Poland
e-mail: kwiat@biol.uni.lodz.pl

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ABSTRACT

The velocity of cytoplasm streaming in an antheridial basal cell and in a subbasal cell as well as in internodal 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 $\mu\text{m/s}$) was observed in a subbasal cell while in a basal cell it was quite intensive during antheridial filament cells proliferation but falling to half of it during spermatozoid differentiation (ca 20 $\mu\text{m/s}$ and 10 $\mu\text{m/s}$ respectively). In internodal cells of pleuridia the velocity was ca 17 $\mu\text{m/s}$.

GA₃ at the 10⁻⁵M concentration decreased the velocity of streaming in a basal cell during proliferation of antheridial filament cells and increased it during spermiogenesis. In internodal 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 multicellular system; it also depends on a developmental stage.

KEY WORDS: cytoplasm streaming, *Chara vulgaris*, basal and subbasal cells, internodal 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 sizes (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 antheridial basal cell joining antheridium and thallus; 2) a subbasal cell in a pleuridium node which occupies a specific position and has plasmodesmal connections with antheridial and oogonial basal cells (Kwiatkowska and Maszewski 1986, unpubl. data); 3) internodal 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 antheridial filament cells, whose stages are marked by the number of cells in antheridial filaments, and during spermiogenesis (differentiation of spermatozoid) which in *Chara vulgaris* starts most often at 64-cell stage. Spermiogenesis is characterised by deep changes in nucleus and in spermatid cytoplasm which made it possible to distinguish 10 spermiogenesis phases in a light microscope (Popłońska 1999; Kwiatkowska and Popłońska 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-

Fig. 1. Schematic drawing illustrating a cross-section of antheridia and oogonia with part of thallus.

--- walls with plasmodesmata, 1 – basal cell of antheridium, 2 – subbasal cell adjoining both gametangia, 3 – internodal cell of pleuridium.

Locality of cytoplasm streaming was examined. GA_3 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, Piłsudskiego 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^{-5} M GA_3 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 filled with pond water with or without GA_3 (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 $p \leq 0.05$.

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 symplically connected with two gametangia: male – antheridium and female – oogonium by their basal cells (Fig. 1). The velocity was about $40 \mu\text{m/s}$ and was roughly the same during all spermatogenesis phases (statistic difference – insignificant) (Fig. 2). The cytoplasm in an antheridial 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 $20 \mu\text{m/s}$ 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 $10 \mu\text{m/s}$.

Experiments with GA_3 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 GA_3 administration rose to ca $50 \mu\text{m/s}$ 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).

— control, - - - - GA_3 .

Fig. 3. Streaming velocity of antheridial basal cell cytoplasm during proliferation (1, 2, 4, 8, 16, 32) and spermiogenesis (I-X).

— control, - - - - GA_3 .

In the light of our previous studies concerning GA_3 role in regulation of morphogenesis in *Chara* (Godlewski and Kwiatkowska 1980; Kwiatkowska and Godlewski 1980; Kaźmierczak et al. 1999) the results seem to indicate that changing reaction of a basal cell to GA_3 was caused by a different level of gibberellins 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 Maszewski 1994). Capillar electrophoresis showed that a proliferative phase in antheridium development was characterised by a very high GA_3 level (Kaźmierczak et al. 1999). Thus it may be suggested that GA_3 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 symplasmic connections between basal cell and antheridium were broken at 16-cell antheridial filament stage (Kwiatkowska 1988) most likely import of GAs from pleuridia decreased, as plasmodesmata were the main routes of GAs transport (Kwiatkowska 1991). The level of endogenous gibberellins in antheridium dropped significantly (Kaźmierczak et al. 1999) probably leading, with some delay to their decrease in a basal cell. In this new physiological situation GA_3 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 internodal cells of pleuridia which react, as it was previously observed, to exogenous GA_3 at the concentration 10^{-5} M with the decrease in metabolic activity expressed by limited elongation (Kwiatkowska and Godlewski 1980). Figure 4 shows that GA_3 at this concentration caused statistically significant decrease in cytoplasm streaming velocity in internodal cells of pleuridia which supports our interpretation of the obtained data concerning basal cells.

Fig. 4. Velocity of cytoplasmic streaming in internodal cells of pleuridia coming from the first and second nodes of main axis of *Chara vulgaris* in the control and after GA_3 10^{-5} M administration.

Different character of streaming in a subbasal cell as compared to an antheridial basal cell may be due to its specific position between two gametangia characterised with different developmental rate. For monoecious species such as *Chara* „protandria” – 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 symplasmic 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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LITERATURE CITED

- ACKERS D., BUCHEN B., HEJNOWICZ Z., SIEVERS A. 2000. The pattern of acropetal and basipetal cytoplasmic streaming velocities in *Chara* rhizoids and protonemata, and gravity effect on the pattern as measured by laser-Doppler-velocimetry. *Planta* 211: 133-143.
- BRAUN M., RICHTER P. 1999. Relocation of the calcium gradient and a dihydropyridine receptor is involved in upward bending by bulging of *Chara* protonema, but not in downward bending by bowing of *Chara* rhizoids. *Planta* 209: 414-423.
- BRAUN M., SIEVERS A. 1993. Centrifugation causes adaptation of microfilaments. Studies on the transport of statoliths in gravity sensing *Chara* rhizoids. *Protoplasma* 174: 50-61.
- BRAUN M., WASTENEYS G.O. 1998. Distribution and dynamics of the cytoskeleton in graviresponding protonemata and rhizoids of characean algae: exclusion of microtubules and a convergence of actin filaments in the apex suggest an actin-mediated gravitropism. *Planta* 205: 39-50.
- BUCHEN B., HEJNOWICZ Z., BRAUN M., SIEVERS A. 1991. Cytoplasmic streaming in *Chara* rhizoids. Studies in a reduced gravitational field during parabolic flights of rockets. *Protoplasma* 165: 121-126.
- GODLEWSKI M., KWIATKOWSKA M. 1980. Effect of gibberellic acid on the formation and development of antheridia and oogonia in *Chara vulgaris* L. *Acta Soc. Bot. Pol.* 49: 459-469.
- GUERLESQUIN M. 1959. Action d'une hormone androgène synthétique sur la fructification de *Charopsis Brunii* Kütz (Charophycées). *Bull. Soc. Sc.* 89^c: 193-195.
- HAGASHI-FUJIME S., ISHIKAWA R., IWASAWA H., KAGAMI O., KURIMOTO E., KOHAMA K., HOZUMI T. 1995. The fastest actin – based motor protein from the green algae, *Chara*, and its distinct mode of interaction with actin. *FEBS Letters* 375: 151-154.
- HEJNOWICZ Z., BUCHEN B., SIEVERS A. 1985. The endogenous difference in the rates of acropetal and basipetal cytoplasmic streaming in *Chara* rhizoids is enhanced by gravity. *Protoplasma* 125: 219-229.

- KAMIYA N. 1986. Cytoplasmic streaming in giant algal cells: A historical survey of experimental approaches. *Bot. Mag. Tokyo* 99: 441-467.
- KAZMIERCZAK A., KWIATKOWSKA M., POPŁOŃSKA K. 1999. GA_3 content in antheridia of *Chara vulgaris* at the proliferative stage and in spermiogenesis estimated by capillary electrophoresis. *Folia Histochem. Cytobiol.* 37: 49-52.
- KURODA K. 1990. Cytoplasmic streaming in plant cells. *Int. Rev. Cytol.* 121: 267-307.
- KWIATKOWSKA M. 1991. Autoradiographic studies on the role of plasmodesmata in the transport of gibberellin. *Planta* 183: 294-299.
- KWIATKOWSKA M. 1995. Effect of symplasmic isolation and antigibberellin treatment on morphogenesis in *Chara*. *Folia Histochem. Cytobiol.* 33: 133-137.
- KWIATKOWSKA M., GODLEWSKI M. 1980. Effect of gibberellic acid and AMO-1618 on the development of vegetative systems in generatively matured thalli of *Chara vulgaris* L. *Acta Soc. Bot. Pol.* 49: 445-458.
- KWIATKOWSKA M., GODLEWSKI M. 1988. Studies on the role of gibberellins in the regulation of spermatogenesis in *Chara vulgaris* L. *Acta Soc. Bot. Pol.* 57: 547-553.
- KWIATKOWSKA M., MASZEWSKI J. 1986. Changes in occurrence and ultrastructure of plasmodesmata in antheridia of *Chara vulgaris* L. during different stages of spermatogenesis. *Protoplasma* 132: 179-188.
- KWIATKOWSKA M., POPŁOŃSKA K. 2002. Further ultrastructural research of *Chara vulgaris* spermiogenesis: Endoplasmic reticulum, structure of chromatin, ^3H -lysine and ^3H -arginine incorporation, *Folia Histochem. Cytobiol.* 40: 85-97.
- KWIATKOWSKA M., PALA A., MASZEWSKI J. 1997. Size-variation in the antheridia and oogonia of *Chara vulgaris* under different experimental conditions. *Acta Soc. Bot. Pol.* 1: 29-32.
- MALINOWSKI S., MASZEWSKI J. 1994. DNA endoreduplication, RNA and protein synthesis during growth and development of the antheridial basal cell in *Chara vulgaris* L. *Folia Histochem. Cytobiol.* 32: 137-142.
- NAGAI R. 1993. Regulation of intracellular movements in plant cells by environmental stimuli. *Int. Rev. Cytol.* 145: 251-309.
- POPŁOŃSKA K. 1999. Cytochemical, autoradiographic and ultrastructural studies the process of spermiogenesis *Chara sp.* – doctor's thesis (in Polish) Univ. of Łódź
- SCHULTE C., KIRST G.O., WINTER U. 1994. Source-sink characteristic of photoassimilate transport in fertile and sterile plants of *Chara vulgaris* L. *Bot. Acta* 107: 362-368.
- SIEVERS A., KRAMER-FISCHER M., BRAUN M., BUCHEN B. 1991. The polar organisation of the growing *Chara* rhizoid and the transport of statoliths are actin-dependent. *Bot. Acta* 104: 103-109.
- STAVES M.P. 1997. Cytoplasmic streaming and gravity sensing in *Chara* internodal cells. *Planta* 203: S79-S84.
- YAMAMOTO K., KIKUYAMA M., SUTOH-YAMAMOTO N., KAMITSUBO E., KATAYAMA E. 1995. Myosin from alga *Chara*: Unique structure revealed by electron microscopy. *J. Mol. Biol.* 254: 109-112.

PRĘDKOŚĆ RUCHU CYTOPLAZMY
W KOMÓRCIE BAZALNEJ I SUBBAZALNEJ ANTERYDIUM
JAK RÓWNIEŻ W KOMÓRKACH INTERNODALNYCH PLEURYDIUM
U *CHARA VULGARIS* L. ORAZ WPLYW GA_3 NA TEN PROCES

STRESZCZENIE

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

GA_3 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 GA_3 obniżeniu ulega także prędkość ruchu w komórkach internodalnych pleurydiów, natomiast w komórce subbasalnej 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 subbasalna, komórki internodalne.