Dependency of Cytoplasmic Streaming on Intracellular ATP and Mg^{2+} Concentrations

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ABSTRACT. The rate of cytoplasmic streaming (v) was measured with reference to intracellular ATP and Mg^{2+} concentrations in tonoplast-free cells prepared by replacing the cell sap with EGTA-containing medium. The relation of v versus $[ATP]_i$ was a rectangular hyperbola. The ATP concentration for half saturation of v was 0.08 mM in *Nitella axillaris* and 0.06 mM in *Chara corallina*. From the results it is concluded that ATP is saturated for cytoplasmic streaming in normal cells, since the ATP concentration in the normal cytoplasm is about 0.5 mM. The relation between $[Mg^{2+}]_i$ and v differed from that between $[ATP]_i$ and v . Stable cytoplasmic streaming was observed only when $[Mg^{2+}]_i$ was equal to or higher than $[ATP]_i$. The inhibition of cytoplasmic streaming by depletion of ATP was completely reversible but that by depletion of Mg^{2+} was irreversible. ADP supported streaming. This may be explained by assuming that ATP was synthesized from ADP by the action of adenyl kinase in the cell.

The motive force of cytoplasmic streaming in Characeae cells is generated at the boundary between the stationary cortical gel layer and the mobile sol endoplasm (the sol-gel interface). The nature of the motive force is a shearing force produced by the organized gel surface and the sol phase (6). Bundles of microfilaments situated at the surface of the gel phase are assumed to be the component responsible for the generation of the motive force (4, 5, 12). The filament binds rabbit muscle heavy meromyosin in arrowhead arrays, which indicates that the filaments are F-actin (14, 15, 23). The relationship between the direction of the arrowheads and that of cytoplasmic streaming has also been clarified (9). Chen and Kamiya (2), and Kuroda and Kamiya (11) showed evidence suggesting that another (putative) component, myosin, is localized in the sol layer. Kato and Tonomura (8) purified myosin from *Nitella flexillis*.

The so-called cell model is a suitable system for determining the mechanism of cell motility; including muscle contraction (19), flagella and cilia movements (1, 13), amoeboid movement (18), movement of myxomycete plasmodium (7) and egg cleavage (10). Williamson (24) removed tonoplasts by perfusing the vacuole rapidly with a medium containing EGTA, a Ca^{2+} -chelator. Tazawa, Kikuyama and Shimmen (21)

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^{b)} Abbreviations: ADP, adenosine-5'-diphosphoric acid; AMP, adenosine-5'-monophosphoric acid; ATP, adenosine-5'-triphosphoric acid; CyDTA, cyclohexanediamine-N,N,N',N'-tetraacetic acid; EGTA, glycoetherdiamine-N,N,N',N'-tetraacetic acid; PEP, phosphoenol pyruvic acid

prepared tonoplast-free cells by replacing the cell sap with a medium containing EGTA. The tonoplast-free cell serves as a good model for studying cytoplasmic streaming, because the chemical composition around the sol-gel interface can be easily controlled. Williamson (24) and Tazawa et al., (21) showed that in tonoplast-free cells both ATP and Mg^{2+} are essential for streaming. In the *Nitella* internode the isolated endoplasmic drop whose surface membrane is removed (11) can also be a suitable model for studying the rotational movement of chloroplasts in the endoplasm.

Relationships between the rate of cytoplasmic streaming and the intracellular ATP and Mg^{2+} concentrations were studied using tonoplast-free cells.

MATERIALS AND METHODS

Nitella axillaris cultured in the laboratory was used except when noted. In the latter case *Chara corallina* cultured outdoors was used. Internodal cells were separated from neighboring cells and kept in tap water before use.

Compositions of the intracellular perfusion media are shown in Table 1. EGTA, K^+ , Tris-maleate and sorbitol were added to all perfusion media. The concentrations of ATP and Mg^{2+} were 0–1 mM and 0–6 mM, respectively. The characterization of the perfusion medium was made by showing the concentrations of ATP and Mg^{2+} . When the medium contained 1 mM ATP and 6 mM Mg^{2+} , it was called 1ATP6Mg medium. The osmotic value of the medium was adjusted to about the equivalent of 330 mM sorbitol by controlling the sorbitol concentration. In some cases the ATP concentration in the medium was buffered by adding PEP and pyruvate kinase to the medium.

The method of producing tonoplast-free cells by vacuolar perfusion has been described elsewhere (21). After the cell sap was replaced with the perfusion medium, the cell was ligated at both opened ends with strips of polyester thread then bathed in artificial pond water (APW) containing 0.1 mM each of KCl, NaCl and $CaCl_2$. Fig. 1 shows the internal morphologies of internodal cells. After the completion of vacuolar perfusion, there were no longer granules in the vacuole (Fig. 1b). After disintegration of the tonoplast, a large part of the endoplasm dispersed into the original vacuolar space and a smaller part moved along the chloroplast layer (Fig. 1c). The speed of a granule (v) was obtained by measuring the time needed to migrate a distance 25–200 μm . Speeds of at least the three fastest granules were measured and the average value was used as the rate of cytoplasmic streaming.

To control the intracellular ATP and Mg^{2+} concentrations, the second and third perfusions were carried out after the tonoplast had been removed by the first perfusion with the EGTA-containing medium. In the first perfusion, replacement of the cell sap with artificial medium was confirmed by the effusion of cell sap which had been stained with neutral red beforehand

TABLE 1. COMPOSITIONS OF PERFUSION MEDIA

	concentration (mM)
ATP	0–1
$MgCl_2$	0–6
EGTA	5
K^+	18–22
Tris-maleate	5
sorbitol	275–290
pH	7

Fig. 1. Longitudinal section of an internodal cell before and after disintegration of the tonoplast. cw; cell wall, pl; plasmalemma, tp; tonoplast, vac; vacuole, en; flowing endoplasm, s-g; sol-gel interface where the motive force is generated, chl; stationary chloroplast layer to which the actin bundles are anchored. a. Normal cell; before vacuolar perfusion, granules were observed in the vacuole. b. Vacuolar perfusion; after the cell sap had been replaced with artificial medium, there were no granules in the vacuole. c. After 1st perfusion; after disintegration of the tonoplast due to replacement of the cell sap with EGTA-containing medium, the original vacuolar space was filled with a mixture of sol endoplasm and the first perfusion medium. The endoplasm attached to the gel layer showed active streaming. d. After 2nd perfusion; a mixture of the sol components and the first perfusion medium which had filled the original vacuolar space was effused by this second perfusion. The endoplasm remaining in the cell moved along the chloroplast layer, when the second perfusion medium contained enough ATP and Mg^{2+} .

(20). In the second or third perfusion the cell interior was perfused with an amount of medium about three times that of the cell volume. In the second perfusion internal fluid containing many endoplasmic fragments was effused and only a minor amount of sol components remained in the cell and moved along the files of chloroplasts (Fig. 1d). After the end of the second perfusion only 1% of the diffusible substances originally present in the cell remained unexchanged (17).

Experiments were conducted at room temperature (18–25°C). Mean value of the rate of cytoplasmic streaming is shown with SEM and number of cells used (n).

RESULTS

The rate of cytoplasmic streaming (v) in relation to the intracellular ATP concentration ($[ATP]_i$). The rate of cytoplasmic streaming for normal cells of *N. axillaris* was $100 \pm 3 \mu\text{m/sec}$ ($n=6$) at 19°C. To prepare tonoplast-free cells, the cell sap was replaced with OATP6Mg medium. After both open cell ends were closed, the cell was kept in APW. The streaming rate after distinegration of the tonoplast was $45 \pm 6 \mu\text{m/sec}$ ($n=8$). The concentration of free Ca^{2+} ($[Ca^{2+}]_i$) in the cell after disintegration of the tonoplast is estimated to be very low (1.3×10^{-8} M, 21). A very low $[Ca^{2+}]_i$ does not affect cytoplasmic streaming but a high $[Ca^{2+}]_i$ causes decreases in v (24). The tonoplast-free cells were perfused for the second time with media containing 6 mM Mg^{2+}

Fig. 2. The rate of cytoplasmic streaming (v) versus $[ATP]_i$. ○; $[ATP]_i$ was not buffered. ●; $[ATP]_i$ was buffered by PEP and pyruvate kinase. Three to five cells were used for measurement of v at each $[ATP]_i$. The average rate is shown with $\pm$ SEM (vertical line). Measurement of v was carried out at $19 \pm 1^\circ\text{C}$.

and various concentrations of ATP (0–1 mM). The number of moving granules decreased with time, because traveling granules often stopped at the ends of the cell. Consequently, v was measured soon after the cell was ligated at the end of the second perfusion. The relation between $[ATP]_i$ and v is shown in Fig. 2. Because physiological activities requiring ATP in tonoplast-free cells, such as cytoplasmic streaming (21, 24) and electrogenic pump(s) (16), continue, $[ATP]_i$ would decrease with time, if there is not sufficient synthesis of ATP after disintegration of the tonoplast. To avoid possible changes in ATP-concentration below 0.25 mM, $[ATP]_i$ was buffered with 1 mM PEP and 1 mg/ml pyruvate kinase. PEP, itself, could not fuel streaming. At higher concentrations, 0.5 and 1.0 mM, the ATP concentration was not buffered, since at these concentrations ATP was saturated for streaming. The rate of streaming was increased by buffering $[ATP]_i$ (Fig. 2). The relation between $[ATP]_i$ and v was a rectangular hyperbola. $K_{1/2}$, which is $[ATP]_i$ where v is half the maximum rate, was 0.08 mM.

In *Chara corallina* the v of cells containing 1 ATP6Mg medium was 60 $\mu\text{m}/\text{sec}$ and $K_{1/2}$ was 0.06 mM at 19°C (data not shown).

The rate of cytoplasmic streaming in relation to the intracellular Mg^{2+} concentration ($[\text{Mg}^{2+}]_i$). The relation between $[\text{Mg}^{2+}]_i$ and v was investigated. After removal of the tonoplast by the first perfusion with 0 ATP6Mg medium, medium containing 1 mM ATP and various concentrations of MgCl_2 was introduced into the cell. $[ATP]_i$ was not buffered. The rate of cytoplasmic streaming was measured about one minute

TABLE 2. RELATION BETWEEN $[Mg^{2+}]_i$ AND THE STREAMING RATE (v) IN CELLS CONTAINING 1 mM ATP

Medium						
1ATP0Mg	1ATP0.05Mg	1ATP0.1Mg	1ATP0.25Mg	1ATP0.5Mg	1ATP1Mg	1ATP6Mg
0	0	42	0*	34	59	80
0	0	0	0	42	67	83
0	0	0	1	0*	63	74
	0*	0	0	50		
	20	0	0	0		
	9	9	0	4		
0				22±9	63±2	79±3

* Moving granules were observed but v could not be measured, because granules stopped soon after microscopic observation was began. Experiments were done at $21 \pm 2^\circ\text{C}$. v is shown in $\mu\text{m}/\text{sec}$.

after the end of perfusion. The relation between $[Mg^{2+}]_i$ and v is shown in Table 2. Between 0 and 0.25 mM $[Mg^{2+}]_i$, in most cells no moving granules were observed. At 0.5 mM $[Mg^{2+}]_i$, v varied significantly among cells. Some granules stopped soon after observation. At 1 mM $[Mg^{2+}]_i$, stable cytoplasmic streaming was observed (63 $\mu\text{m}/\text{sec}$). The rate increased when $[Mg^{2+}]_i$ was increased to 6 mM. Cytoplasmic streaming was observed even at an $[Mg^{2+}]_i$ lower than 0.5 mM, when $[ATP]_i$ was decreased to the same level as $[Mg^{2+}]_i$ (Table 3).

The reversibility of cytoplasmic streaming for changes in $[ATP]_i$ or $[Mg^{2+}]_i$. The recovery of v due to the introduction of ATP into ATP-depleted cells was tested (Table 4A). The rate of streaming in the tonoplast-free cell prepared by the first perfusion with 1ATP6Mg medium was $87 \pm 5 \mu\text{m}/\text{sec}$. Variations in v among the cells (Table 4) seem to have resulted from uncontrolled room temperature (18 – 25°C). The cell interior was perfused with 0ATP6Mg medium. The depletion of ATP resulted in the cessation of cytoplasmic streaming. When 1ATP6Mg medium was reintroduced into the ATP-depleted cells, v recovered to a level ($80 \pm 4 \mu\text{m}/\text{sec}$) which was nearly equal to the original rate.

The recovery of v due to the introduction of Mg^{2+} into Mg^{2+} -depleted cells was also tested (Table 4B). When the cell interior was perfused with 1ATP0Mg medium

TABLE 3. THE RATE OF CYTOPLASMIC STREAMING (v) IN CELLS CONTAINING ATP AND Mg^{2+} AT THE SAME CONCENTRATIONS

Medium	
0.25ATP0.25Mg	0.5ATP0.5Mg
42	43
45	56
40	48
38	
45	
42±1	49±4

After disintegration of the tonoplast due to replacement of the cell sap with 0ATP6Mg medium, the cell interior was perfused with 0.25ATP0.25Mg or 0.5ATP0.5Mg medium. $[ATP]_i$ was not buffered. Experiments were done at $21 \pm 2^\circ\text{C}$, v is shown in $\mu\text{m}/\text{sec}$.

TABLE 4. REVERSIBILITY OF THE RATE OF CYTOPLASMIC STREAMING (v in $\mu\text{m}/\text{sec}$)
DUE TO CHANGES IN $[\text{ATP}]_i$ AND $[\text{Mg}^{2+}]_i$

A				B			
Cell No.	Perfusion medium			Cell No.	Perfusion medium		
	First 1ATP6Mg	Second 0ATP6Mg	Third 1ATP6Mg		First 1ATP6Mg	Second 1ATP0Mg	Third 1ATP6Mg
1	71	0	63	1	61	0	0
2	69	0	77	2	61	0	0
3	95	0	83	3	91	0	0
4	95	0	91	4	91	0	0
5	91	0	87	5	93	0	0
6	100	0	80	Average	79 ± 8	0	0
Average	87 ± 5	0	80 ± 4				

C				D			
Cell No.	Perfusion medium			Cell No.	Perfusion medium		
	First 1ATP6Mg	Second 0ATP0Mg	Third 1ATP6Mg		First 1ATP6Mg	Second 0ATPCyDTA	Third 1ATP6Mg
1	—	0	11	1	91	—	0
2	91	0	17	2	95	—	0
3	95	0	20	3	95	—	0
4	—	0	11	4	95	—	0
5	80	0	0	Average	94 ± 1	—	0
6	95	0	0				
7	80	0	26				
Average	88 ± 3	0	12 ± 4				

Experiments were done at 18–25°C.

after the tonoplast had been removed by replacing the cell sap with 1ATP6Mg medium, no streaming was observed. Cytoplasmic streaming was not recovered by introducing Mg^{2+} into Mg^{2+} -depleted cells by perfusion with 1ATP6Mg medium. Cytoplasmic streaming, which had stopped after the second perfusion with 0ATP0Mg medium, recovered only slightly after the introduction of ATP and Mg^{2+} (Table 4C). Five minutes elapsed between the second and third perfusions.

$[\text{Mg}^{2+}]_i$ was severely lowered by CyDTA which combines strongly with both Ca^{2+} and Mg^{2+} (Table 4D). After disintegration of the tonoplast by the first perfusion with 1ATP6Mg medium, the cell interior was perfused with 0ATPCyDTA medium containing 5 mM CyDTA, 5 mM Tris-maleate, 21 mM KOH and 305 mM sorbitol (pH 7). The cell was kept on the perfusion bench (21) without closing the open cell ends after the finish of the second perfusion with 0ATPCyDTA medium. One minute after the end of the second perfusion, the cell interior was perfused with 1ATP6Mg medium. Even though the period during which the cell was exposed to a very low $[\text{Mg}^{2+}]_i$ was short (one minute), cytoplasmic streaming did not recover with the introduction of Mg^{2+} into the cell (Table 4D).

Effects of other phosphorous compounds on cytoplasmic streaming. Effects of AMP, ADP, orthophosphate and pyrophosphate on cytoplasmic streaming were tested. After the tonoplast had been removed by replacing the cell sap with 0ATP6Mg medium, the cell interior was perfused with a medium containing 1 mM of the test

Fig. 3. Time course for the rate of cytoplasmic streaming (v) after the introduction of ADP into the tonoplast-free cell. Medium containing 1 mM ADP and 6 mM Mg^{2+} was introduced into the cell after disintegration of the tonoplast caused by the first perfusion with 0ATP6Mg medium. v was measured at 20°C.

compound instead of 1 mM ATP in 1ATP6Mg medium. AMP, orthophosphate and pyrophosphate could not support cytoplasmic streaming, but ADP could. A typical example is shown in Fig. 3. The measurement of v was started about one minute after the end of perfusion with the ADP-containing medium. The rate was low at the beginning and increased with time.

DISCUSSION

The relation between $[ATP]_i$ and the rate of cytoplasmic streaming was a rectangular hyperbola. The value for $K_{1/2}$ was 0.08 mM in *N. axillaris* and 0.06 mM in *C. corallina*. Since the ATP concentration in the cytoplasm of normal Characeae cells is around 0.5 mM (3, Kikuyama, Hayama, Fujii and Tazawa, unpublished), we concluded that the $[ATP]_i$ in normal cells is saturated for the effect on cytoplasmic streaming.

The rate of streaming in the cell with 1ATP6Mg medium was 69 $\mu\text{m}/\text{sec}$ (Fig. 2), which is 69% of the normal rate (100 $\mu\text{m}/\text{sec}$). The decrease in the K^+ concentration to 1/5 (22 mM, Table 1) the normal concentration in the cytoplasm (112 mM, 22) might cause a decrease in v . However, even when the cell sap had been replaced with 1ATP6Mg medium whose K^+ concentration had been increased to 100 mM by adding K-methane sulfonate, after the disintegration of the tonoplast v was 77% of the normal rate.

Because both ATP and Mg^{2+} are responsible for cytoplasmic streaming (21, 24),

a rectangular hyperbola is expected between $[Mg^{2+}]_i$ and v . However, the relation was not a hyperbola (Table 2). Cytoplasmic streaming was almost completely inhibited, when $[Mg^{2+}]_i$ was lower than $[ATP]_i$.

In contrast to the reversible inhibition of cytoplasmic streaming due to the depletion of ATP (Table 4A), the depletion of Mg^{2+} irreversibly inhibited cytoplasmic streaming (Table 4B). Because $[Mg^{2+}]_i$ was lowered by perfusing the cell with 1ATP-0Mg medium (Table 4B), the possibility is that free ATP, which is not in the form of $Mg \cdot ATP$, inhibits cytoplasmic streaming. If this is the case, then the inhibition of cytoplasmic streaming with 0ATP0Mg medium containing no free ATP is reversible. However, only the partial recovery of cytoplasmic streaming was observed when 1ATP6Mg medium was introduced into the cell containing 0ATP0Mg medium (Table 4C). This suggests that this possibility is not valid and that the cause of the irreversible inhibition of streaming caused by 1ATP0Mg medium is the depletion of Mg^{2+} . The involvement of Mg^{2+} ions *per se* in cytoplasmic streaming is further supported by the fact that CyDTA, an extremely potent Mg^{2+} -chelator, inhibited streaming completely in the absence of ATP (Table 4D). The deviation in the relationship between $[Mg^{2+}]_i$ and v from a rectangular hyperbola (Table 2) can be accounted for in terms of the depletion of free Mg^{2+} at an $[Mg^{2+}]_i$ lower than the $[ATP]_i$ (1 mM). Williamson (24) also reported that motility requires a millimolar Mg^{2+} level but he did not examine the effect of free ATP. The necessity for free Mg^{2+} for streaming and the irreversible inhibition of streaming caused by the depletion of Mg^{2+} were demonstrated.

Although the mechanism for the irreversible inhibition of cytoplasmic streaming caused by the depletion of free Mg^{2+} is not clear, Mg^{2+} may have a role in maintaining some structure(s) necessary for streaming as well as a role as cofactor in the ATPase reaction. The fact that the inhibition of cytoplasmic streaming with medium lacking an Mg^{2+} -chelator was only partially reversed by 1ATP6Mg medium (Table 4C) suggests that the association of Mg^{2+} with the 'site' of a structure(s) responsible for the maintenance of cytoplasmic streaming is relatively weak.

Of the phosphorous compounds other than ATP, only ADP was effective (Fig. 3). This coincides with the results obtained by Williamson (24). The rate increased with time after intracellular perfusion with medium containing 1 mM ADP and reached $40 \mu\text{m}/\text{sec}$ (Fig. 3). The increase in v with time is believed to result from the synthesis of ATP from ADP due to the action of adenyl kinase in the cell (16, 24). The ATP concentration in the cell 15 minutes after the introduction of ADP was calculated to be 0.074 mM from Fig. 1.

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REFERENCES

1. BROKAW, C.J. Movement of the flagella of *Polytoma uvella*. *J. Exp. Biol.* **40**, 149–156, 1963
2. CHEN, J.C.W. and N. KAMIYA. Localization of myosin in the internodal cell of *Nitella* as suggested by differential treatment with N-ethylmaleimide. *Cell Structure Function* **1**, 1–9, 1975
3. HATANO, S. and H. NAKAJIMA. ATP content and ATP-dephosphorylating activity of *Nitella*. *Ann. Rep. Sci. Works Fac. Sci. Osaka Univ.* **11**, 71–76, 1963
4. KAMITSUBO, E. Motile protoplasmic fibrils in cells of Characeae. II. Linear fibrillar structure

- and its bearing on cytoplasmic streaming. *Proc. Japan Acad.* **42**, 640–643, 1966
5. KAMITSUBO, E. Motile protoplasmic fibrils in the cells of Characeae. *Protoplasma* **74**, 53–70, 1972
 6. KAMIYA, N. and K. KURODA. Velocity distribution of the protoplasmic streaming in *Nitella* cells. *Bot. Mag. Tokyo* **69**, 543–554, 1956
 7. KAMIYA, N. and K. KURODA. Movement of myxomycete plasmodium. I. A study of glycerinated models. *Proc. Japan Acad.* **41**, 837–841, 1965.
 8. KATO, T. and Y. TONOMURA. Identification of myosin in *Nitella flexilis*. *J. Biochem.* **82**, 777–782, 1977
 9. KERSEY, Y.M., P.K. HEPLER, B.A. PALEVITZ and N.K. WESSELLS. Polarity of actin filaments in characean algae. *Proc. Natl. Acad. Sci. USA.* **73**, 165–167, 1976
 10. KINOSHITA, S. and I. YAZAKI. The behavior and localization of intracellular relaxing system during cleavage in the sea urchin egg. *Exp. Cell Res.* **47**, 449–458, 1967
 11. KURODA, K. and N. KAMIYA. Active movement of *Nitella* chloroplasts *in vitro*. *Proc. Japan Acad.* **51**, 774–777, 1975
 12. NAGAI, R. and L.I. LEBHAN. Cytoplasmic microfilaments in streaming *Nitella* cells. *J. Ultrastruct. Res.* **14**, 571–589, 1966
 13. NAITOH, Y. and H. KANEKO. Reactivated Triton-extracted models of *Paramecium*: Modification of ciliary movement by calcium ions. *Science* **176**, 523–524, 1972
 14. PALEVITZ, B.A., J.F. ASH and P.K. HEPLER. Actin in the green alga *Nitella*. *Proc. Natl. Acad. Sci. USA.* **71**, 363–366, 1975
 15. PALEVITZ, B.A. and P.K. HEPLER. Identification of actin *in situ* at the ectoplasm-endoplasm interface of *Nitella*. Microfilament-chloroplast association. *J. Cell Biol.* **65**, 29–38, 1975
 16. SHIMMEN, T. and M. TAZAWA. Control of membrane potential and excitability of *Chara* cells with ATP and Mg^{2+} . *J. Membrane Biol.* **37**, 167–192, 1977
 17. SHIMMEN, T. and M. TAZAWA. Intracellular chloride and potassium ions in relation to excitability of *Chara* membrane. (submitted to *J. Membrane Biol.*)
 18. SIMARD-DUQUESNE, N. and P. COUILLARD. Ameboid movement. I. Reactivation of glycerinated models of *Amoeba proteus* with adenosine triphosphate. *Exp. Cell Res.* **28**, 85–91, 1962
 19. SZENT-GYÖRGI, A. Free-energy relations and contraction of muscle. *Biol. Bull.* **96**, 140–161, 1949
 20. TAZAWA, M. Studies on *Nitella* having artificial cell sap. I. Replacement of the cell sap with artificial solutions. *Plant Cell Physiol.* **5**, 33–43, 1964
 21. TAZAWA, M., M. KIKUYAMA and T. SHIMMEN. Electric characteristics and cytoplasmic streaming of Characeae cells lacking tonoplast. *Cell Structure Function* **1**, 165–176, 1974
 22. TAZAWA, M., U. KISHIMOTO and M. KIKUYAMA. Potassium, sodium and chloride in the protoplasm of Characeae. *Plant Cell Physiol.* **15**, 103–110, 1976
 23. WILLIAMSON, R.E. Actin in the alga *Chara corallina*. *Nature (Lond.)* **248**, 801–802, 1974
 24. WILLIAMSON, R.E. Cytoplasmic streaming in *Chara*: A cell model activated by ATP and inhibited by cytochalasin B. *J. Cell Sci.* **17**, 655–668, 1975

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