

Gravity-dependent polarity of cytoplasmic streaming in *Nitellopsis*

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Summary. The internodal cells of the characean alga *Nitellopsis obtusa* were chosen to investigate the effect of gravity on cytoplasmic streaming. Horizontal cells exhibit streaming with equal velocities in both directions, whereas in vertically oriented cells, the downward-streaming cytoplasm flows ca. 10% faster than the upward-streaming cytoplasm. These results are independent of the orientation of the morphological top and bottom of the cell. We define the ratio of the velocity of the downward- to the upward-streaming cytoplasm as the polar ratio (PR). The normal polarity of a cell can be reversed (PR < 1) by treatment with neutral red (NR). The NR effect may be the result of membrane hyperpolarization, caused by the opening of K⁺ channels. The K⁺ channel blocker TEA Cl⁻ inhibits the NR effect.

External Ca²⁺ is required for normal graviresponsiveness. The [Ca²⁺] of the medium determines the polarity of cytoplasmic streaming. Less than 1 μM Ca²⁺ resulted in a PR < 1 while greater than 1 μM Ca²⁺ resulted in the normal gravity response. The voltage-dependent Ca²⁺-channel blocker, nifedipine, inhibited the gravity response in a reversible manner, while treatment with LaCl₃ resulted in a PR < 1, indicating the presence of two types of Ca²⁺ channels. A new model for graviperception is presented in which the whole cell acts as the gravity sensor, and the plasma membrane acts as the gravireceptor. This is supported by ligation and UV irradiation experiments which indicate that the membranes at both ends of the cell are required for graviperception. The density of the external medium also affects the PR of *Nitellopsis*. Calculations are presented that indicate that the weight of the protoplasm may provide enough potential energy to open ion channels.

Keywords: Ca²⁺; Cytoplasmic streaming; Gravity; *Nitellopsis obtusa*; Polarity.

Introduction

It is fascinating to consider the mechanisms involved in the perception of gravity in plants. The force of gravity is the weakest of the fundamental forces (strong

force, weak force, electromagnetic force and gravitational force), yet it incessantly acts on plants, influencing many developmental events from cellular stratification and the distribution of mRNA to the polarity of the root/shoot axis (McClure and Guilfoyle 1989). Sedimenting statoliths, including amyloplasts and BaSO₄ crystals are considered the most likely candidates for the gravireceptor (Sievers and Volkmann 1979), however, there are a number of gravity sensing systems that do not have statoliths (Dennison and Shropshire 1984, Ewart 1903, Pickard and Thimann 1966). In these cases, the plasma membrane may act as the gravireceptor. While it is likely that many different gravireceptors have evolved for the sensory detection of gravity in varied ecological environments and in each evolutionarily distinct organism, it is also possible that the plasma membrane is a common gravireceptor in all organisms, with statoliths acting as antennae that increase the sensitivity of the responding cells to gravity. We have chosen the internodal cells of *Nitellopsis* as a model system to study graviperception because it is devoid of any visible statoliths.

Ewart (1903) observed that the polarity of the rotational cytoplasmic streaming in characean cells is responsive to gravity. This was later confirmed by Hayashi (1957). The polarity of circulatory streaming in higher plant cells is also regulated by gravity (Ewart 1903, Bottelier 1934). We presently consider the effect of gravity on cytoplasmic streaming and the intermediate signal transduction and transmission steps as the complete system from the stimulus to the response. We are using the gravity-regulated polarity of cytoplasmic streaming as a tool to understand graviperception and

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signal transduction in plants. However, the effect of gravity on cytoplasmic streaming may participate in gravitropic responses by regulating the distribution of molecules or organelles whose transport is limited by diffusion (Audus 1979, Kessler 1979).

Materials and methods

Plant materials

Nitellopsis obtusa (Desv. in Lois.) J. Gr. was grown in a soil water mixture in large glass tanks at 28 °C with continuous light. Typically, young internodal cells of *Nitellopsis* (4–6 cm) were isolated and placed in artificial pond water (APW: 0.1 mM NaCl, 0.1 mM KCl and 0.1 mM CaCl₂) buffered by 2 mM Hepes-NaOH, pH 7.2 at least overnight before use. Sometimes cells that had a buildup of CaCO₃ crystals on the walls were used. In this case, the cells were first placed in APW buffered by 10 mM Mes titrated with Tris to pH 5.5. The cells remained in this medium overnight and were then transferred to APW buffered with 2 mM Hepes-NaOH, pH 7.2. The cells remained in this medium at least overnight before use. All experiments were carried out at 25 ± 2 °C.

Observation of cells

Cells were placed in a Plexiglas chamber in which the cell ends were embedded in Dow Corning silicone grease. The rest of the cell was bathed in APW buffered with 2 mM Hepes-NaOH with or without inhibitors. The chamber was covered with a cover glass. The chamber was then placed on a Zeiss Standard horizontal microscope equipped with a rotating stage. The optics of the Zeiss horizontal microscope included a Plan 16X objective (N. A. = 0.35), a 2 × optivar and Kpl-W 10 × /18 oculars.

The cells were oriented so that the two opposing streams adjacent to the indifferent zone could be observed simultaneously. Streaming in an area midway along the length of the internode was observed. The velocity of the streaming endoplasm 6–12 (depending on cell geometry) chloroplast rows away from the indifferent zone was measured. Unless stated otherwise, the velocities of the particles in the bulk endoplasm adjacent to the actin bundles were measured. This was accomplished, under our optical conditions, when the chloroplasts were in sharp focus. The cells typically were oriented so that the chloroplast files were parallel to the force of gravity. Therefore the actin bundles were oriented parallel to the force of gravity (Kamitsubo 1972). In *Nitellopsis* the chloroplast bundles form an angle with the axis of the cell which is 5.15° ± 0.73 (n = 10).

Measurement of the velocity of cytoplasmic streaming

The time it took for small endoplasmic particles to flow 137 μm was measured with a Casio Pulsecheck stopwatch with a resolution of 0.01 seconds. Five measurements were made on a single stream and then five more were made on the opposite stream. This was repeated in a rapid fire manner until 25 measurements were made on each side. First the cell was assayed when the actin cables were in the horizontal position. Then the cell was rotated 90° until the actin cables were vertical and again assayed. Finally the cell was rotated a further 90° until the actin cables were again vertical, but in the opposite orientation relative to the first vertical measurement. The cell was assayed again in this position.

The Polar Ratio is defined as the ratio of the velocity of the downward-directed stream to the velocity of the upwardly-directed stream.

Preparation of Ca²⁺/EGTA buffers

Ca²⁺/EGTA buffers were made with the aid of a computer program which calculates the apparent association constants for cations (Wayne 1985). All the buffers included 10 mM EGTA (titrated with NaOH to pH 7.2), 20 mM Hepes (titrated with NaOH to pH 7.2), 0.1 mM NaCl, 0.1 mM KCl, 1 mM free Mg²⁺ (added as MgCl₂) and various concentrations of CaCl₂. The deionized water was prepared by passing centrally available reverse osmosis water sequentially through a Barnstead D0803 High Capacity ion exchange column and a Barnstead D0809 Mixed Bed Ultrapure ion exchange column. The total calcium concentration of this water, as measured with atomic absorption spectrophotometry, was less than 20 nM.

Cellular ligation and ultraviolet irradiation

Cells were ligated adjacent to the node with Singer Super-Strong Polyester Cord Thread following the method of Kamiya and Kuroda (1956). Cells were allowed to sit in APW buffered with 2 mM Hepes-NaOH (pH 7.2) overnight in order to recover from the shock of ligation. However cells observed immediately after ligation to one month afterwards showed identical results (data not shown).

Cells were irradiated through a cover glass with an ultraviolet microbeam that was constructed from a Zeiss HBO 50 bulb and lamp housing, including a condenser and a field diaphragm. The energy fluence rate at the level of the number 1 cover glass was 260 ± 6 (n = 6) W m⁻² as measured with a YSI-Kettering Model 65A Radiometer.

Experiments using the CMS-II centrifuge microscope of the stroboscopic type

Experiments were performed in collaboration with Professor Eji Kamitsubo of the Biological Laboratories, Hitotsubashi University, Kunitachi, Tokyo 186, Japan. Complete descriptions of the CMS centrifuge microscope of the stroboscopic type and the modifications found in the CMS-II can be found in Kamitsubo et al. (1988, 1989). Cells were bathed in APW in a cuvette and then placed in the rotor. The streaming cytoplasm was observed with a Nikon LWD 40 × (N.A. = 0.55) objective lens combined with a Nikon LWD condenser lens (N.A. = 0.65). All centrifuge microscope experiments were performed at temperatures between 20 and 21 °C. Linear regressions were calculated with the aid of MINITAB.

Chemicals

Tetraethylammonium chloride (TEA Cl⁻) was purchased from Aldrich, Nifedipine, LaCl₃ and W-5 were purchased from Sigma, Neutral Red (NR, C. I. 50040, Total Dye Content = 84%) was purchased from Allied Chemical Corporation, R 24571 was purchased from Janssen Pharmaceutica and W-7 (Sigma) was a gift from Dr. A. Jagendorf.

Statistics

All data are expressed as the mean ± the standard error of the mean. In most cases the error bars are smaller than the symbol indicating the position of the mean. Each experiment was conducted independently at least three times with different cells.

Results

The effect of gravity on cytoplasmic streaming

In a horizontally placed internodal cell of *Nitellopsis obtusa*, the velocity of cytoplasmic streaming adjacent

Table 1. Velocity of cytoplasmic streaming

measurement	Streaming velocity ($\mu\text{m s}^{-1}$)					
	Orientation					
	Vertical		Horizontal		Inverted	
	up	down	left	right	up	down
1	97.16	106.20	112.30	100.00	90.73	107.90
2	97.16	103.80	101.50	102.20	95.14	97.86
3	100.00	106.20	103.80	100.70	82.53	100.00
4	98.56	102.20	96.50	103.80	98.56	98.56
5	99.28	98.56	95.14	103.00	83.03	91.33
6	98.56	112.30	93.84	99.28	91.95	103.80
7	100.00	97.16	105.40	109.60	91.33	104.60
8	92.57	108.70	107.00	86.71	93.84	92.56
9	97.16	103.80	96.48	93.84	93.20	104.60
10	108.70	103.80	103.80	102.20	97.86	99.28
11	97.86	93.84	119.10	107.00	94.48	95.80
12	90.73	119.10	87.26	98.56	96.48	99.27
13	92.57	106.20	95.80	103.80	94.48	100.00
14	91.33	105.40	100.00	90.73	88.96	101.50
15	98.56	111.40	87.26	108.70	98.56	102.20
16	100.00	109.60	98.56	95.80	94.48	83.03
17	76.97	94.48	98.56	91.94	94.48	117.10
18	90.13	104.60	85.63	105.40	100.70	105.40
19	96.48	103.00	97.86	94.48	88.96	94.48
20	95.80	101.50	100.70	102.20	97.16	98.56
21	102.20	93.84	100.70	96.48	95.14	122.30
22	95.80	101.50	95.80	101.50	95.80	103.00
23	87.82	96.48	99.28	93.84	98.56	104.60
24	86.16	104.60	96.48	103.80	98.56	110.50
25	96.48	108.70	96.48	100.70	97.85	94.48
\bar{X}	95.52	103.90	99.01	99.85	94.11	101.30
S.D.	6.17	6.09	7.33	5.71	4.59	8.03
S.E.	1.23	1.22	1.47	1.14	0.92	1.61
n	25	25	25	25	25	25
t	4.84		0.54		3.89	
p	<0.001		0.9 > p > 0.5		<0.001	
Polar Ratio	1.0877		L/R = 0.9916 R/L = 1.0084		1.0764	

The time it took for small endoplasmic particles to flow $137\mu\text{m}$ was measured. Five measurements were made on a single stream and then five more were made on the opposite stream. This was repeated in a rapid fire manner for each orientation until 25 measurements were made on each side. First the cell was assayed when the actin cables were in the vertical position. Then the cell was rotated 90° until the actin cables were horizontal and again assayed. Finally the cells as rotated a further 90° until the actin cables were again vertical, but in the opposite orientation relative to the first measurement. The cell was assayed again in this position. The optics of the Zeiss horizontal microscope included a $16\times$ objective, a $2\times$ optivar and $10\times$ oculars. This is a representative cell; more than fifty cells were examined

to the indifferent zone in one direction is equal to the velocity of cytoplasmic streaming in the opposite direction. However if the cell is rotated 90° , the endoplasm in the downwardly-directed stream flows approximately 10 percent faster than the endoplasm in the upwardly-directed stream (Table 1). This leads to

a polarity in streaming, which we define as the polar ratio. The average polar ratio of vertically-positioned cells is 1.110 ± 0.006 ($n = 51$) compared to 1.009 ± 0.003 ($n = 28$) for horizontally-positioned cells. Relative to the horizontal control, the upwardly-directed stream is inhibited and the downwardly-directed stream is stim-

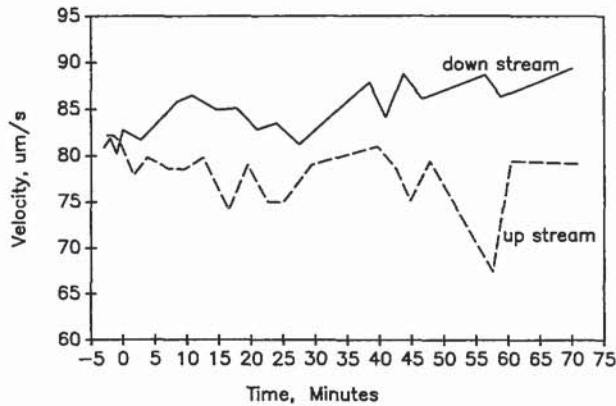


Fig. 1. The time course of the effect of gravity on streaming velocity. At time zero the cell was rotated on the stage of a horizontal microscope. The time resolution is 30 sec. The cell was oriented so that the actin bundles were either perpendicular (-5-0 min) or parallel (0-70 min) to the direction of gravity.

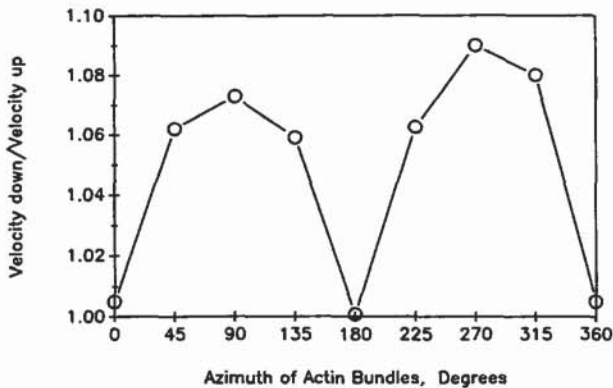
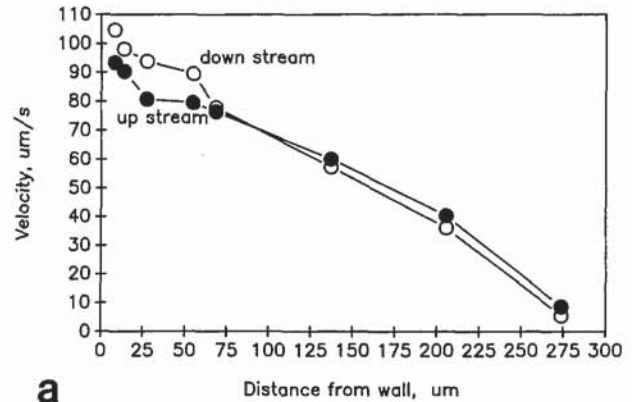
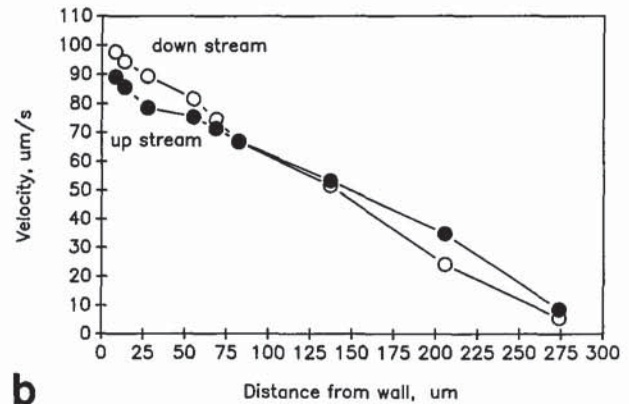


Fig. 2. The effect of the azimuth of the actin bundles with respect to gravity on the magnitude of the response. Cells were rotated 360 degrees on a horizontal microscope and the relative velocity of the downwardly streaming cytoplasm relative to the upwardly streaming cytoplasm was measured.

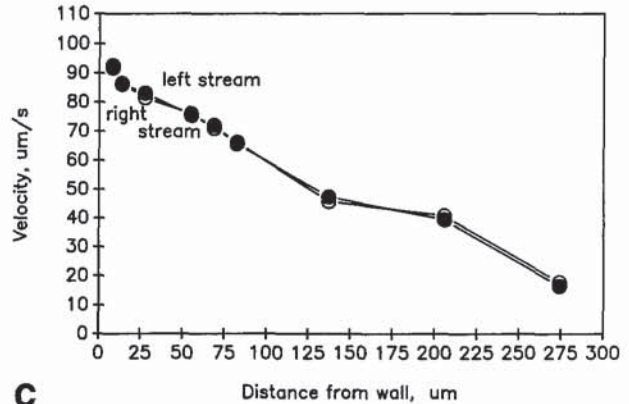
ulated. The magnitudes of the stimulation of the downwardly-directed stream and the retardation of the upwardly-directed stream are identical. There is not any perceptible lag time (Fig. 1, current temporal resolution = 30 sec). The polar ratio is dependent on the azimuth of the indifferent zone (Θ) with respect to gravity (Fig. 2, horizontal orientation = 0°). The magnitude of the response is proportional to the cosine of Θ . Velocity profiles show that gravity has the greatest effect in the peripheral bulk endoplasm near the actin bundles (Fig. 3). Furthermore, the thickness of the retarded upwardly-directed stream is greater than the thickness of the accelerated downwardly-directed stream, indicating that the same volume is being transported up and down and explains why there is not any observable buildup of cytoplasm.



a



b



c

Fig. 3. The velocity distribution of (a) the up and down stream in a vertical cell (90°); (b) the up and down stream of an inverted cell (270°) and (c) the left and right stream of a horizontal cell (0°). Angles refer to the orientation of the actin bundles. Standard errors are smaller than the symbols.

Localization of the site of graviperception and/or signal transduction

We have begun efforts to localize the region of the cell involved in graviperception and/or the early events of signal transduction. Using cellular ligation, we have been able to remove both of the cell ends to test whether the two cell ends, which include nodal cells and plasmodesmata, are necessary for the response. We find

Table 2. The effect of cellular ligation on the polar ratio

Cell number	Polar ratio	
	Intact cell	Ligated cell
1	1.0820	1.0034
2	1.0966	0.9964
3	1.0745	1.0253
4	1.1511	1.0029
5	1.1202	1.0336
\bar{X}	1.1048	1.0123
S.D.	0.0311	0.0161
S.E.	0.0072	0.0139

Cells were left intact or ligated at both ends. The nodes were then removed from ligated cells. Cells remained in APW overnight in order to recover. However, cells measured immediately after ligation yielded identical results

Table 3. The effect of the ligation of a single node on the polar ratio

Treatment	Position		
	normal	horizontal (L/R)	inverted
Control			
Cell 1	1.1577	1.0000	
Proximal node ligated			
Cell 1	0.9836	0.9768	0.9978
Cell 2	0.9719	0.9870	1.0128
Cell 3	0.9886	1.0152	1.0102
\bar{X}	0.9813	0.9930	1.0069
S.D.	0.0086	0.0199	0.0080
S.E.	0.0049	0.0114	0.0046
Distal node ligated			
Cell 1	0.9863	0.9795	1.0315
Cell 2	1.0103	0.9869	0.9984
Cell 3	0.9886	0.9983	0.9806
\bar{X}	0.9950	0.9882	1.0035
S.D.	0.0132	0.0095	0.0258
S.E.	0.0076	0.0054	0.0144

Cells were left intact or ligated at either the morphologically distal end or the morphologically proximal end. The node was then removed from the ligated cells. Cells remained in APW overnight in order to recover. The polar ratio was then tested in the morphologically normal orientation, horizontal orientation and inverted orientation

that cell fragments from which both cell ends have been removed, although streaming normally (streaming rate = $103 \mu\text{m}\cdot\text{s}^{-1}$, $n=29$), can not respond to gravity

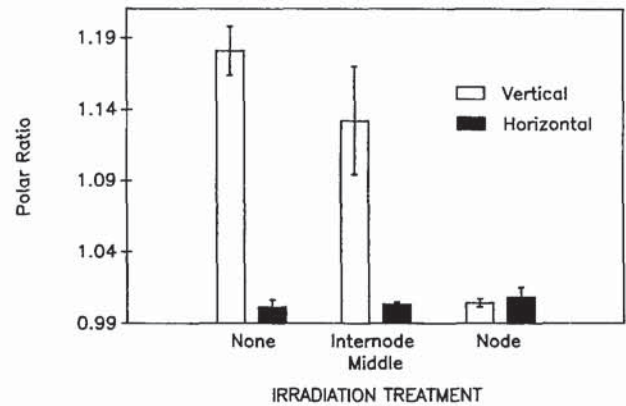


Fig. 4. The effect of site of UV microbeam irradiation on the polar ratio. Cells ($n=3$) were irradiated for 2 min with a 0.5 cm in diameter UV microbeam with an energy fluence rate of 260 W m^{-2} . Cells were either irradiated at an internodal cell end or in the middle of the internode.

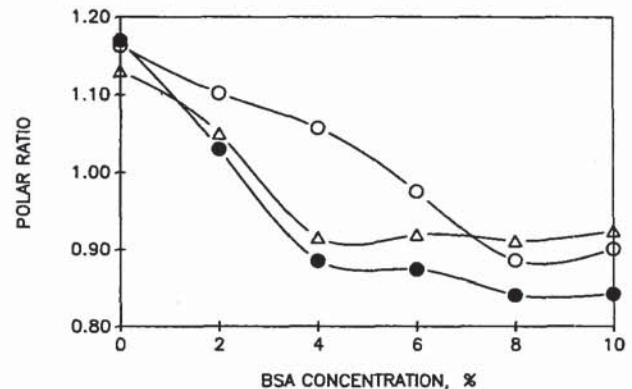


Fig. 5. The effect of Bovine Serum Albumin on the polar ratio. Cells were treated sequentially with increasing concentrations of BSA. Each cell was incubated in increasing concentrations of BSA for 20 minutes. Increasing the incubation time to 1 h did not have any significant effect on the results (data not shown). \circ Cell 1, \bullet cell 2, \blacktriangle cell 3

(Table 2). We also find that internodal cells that possess only one node still are not able to respond to gravity (Table 3). Cells that possess only their distal or proximal node do not respond to gravity irrespective of their orientation.

While ligated cells are completely normal in all respects except for their graviresponsiveness, cellular ligation may disrupt the cell nonspecifically. Therefore we exposed a single cell end to 260 W m^{-2} ultraviolet (UV) light for 2 min. Treatment of the cell end with UV light completely eliminates the gravity-induced polarity of cytoplasmic streaming (Fig. 4). By contrast, treatment of the middle of the cell with UV light has no effect on the gravity-induced polarity. The streaming velocity of UV-irradiated cells was unaltered (velocity = $109 \mu\text{m}\cdot\text{s}^{-1}$, $n=27$). These data indicate that the cell

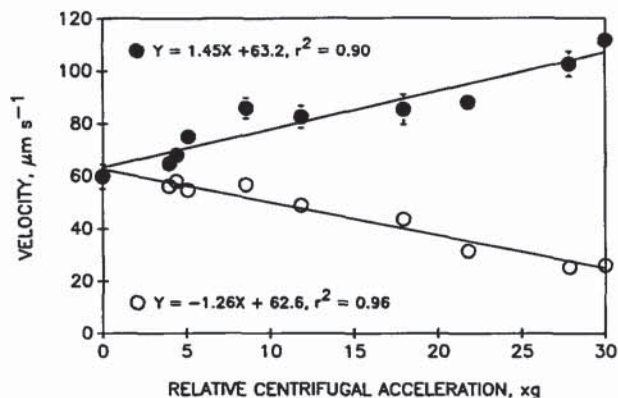


Fig. 6. The effect of centrifugal acceleration on the velocity of the centripetally and centrifugally directed streams of one and the same cell. Streaming was observed with the CMS-II centrifuge microscope of the stroboscopic type. The linear regressions of both lines are shown. The equations of both lines are also shown. Both linear regressions are very highly significant ($P < 0.001$). The interpolated polar ratio of this cell at $1 \times g$ is 1.05. ● Centrifugally directed stream, ○ centripetally directed stream

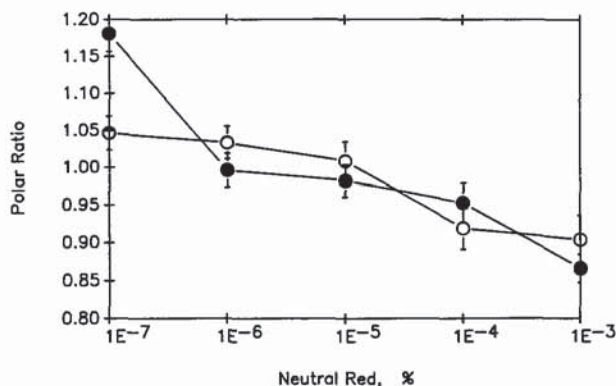


Fig. 7. The effect of neutral red on the polar ratio. The polar ratio is defined as the velocity of the down stream divided by the velocity of the up stream. The standard error bars indicate measurements that were made on the same cell. Cells were incubated in each concentration of neutral red for several hours. ○ Cell 1, ● cell 2. E Exponential notation

ends are specialized regions that participate in the response to gravity. We postulate that the cell ends are involved in graviperception and/or the early events of signal transduction.

The effect of the density of the external medium on the polar ratio

Since the cell ends appear to be required for the graviresponse, we tested the possibility that the falling of the protoplasm *in toto* is required for the realization of the graviresponse. The static buoyancy (B , in New-

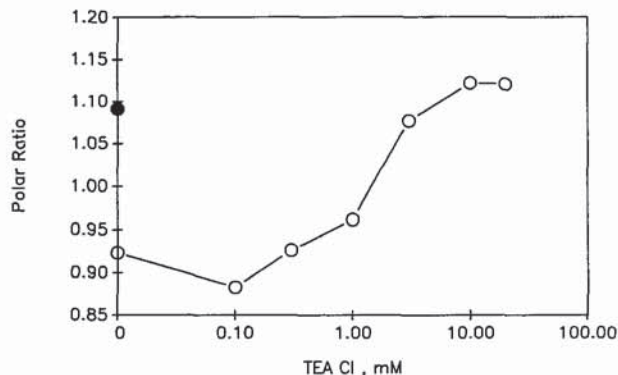


Fig. 8. The effect of TEA Cl^- on the polar ratio of one and the same cell treated with 0.001% neutral red. Closed circle represents the polar ratio of the cell before it was exposed to neutral red. After placement in neutral red, the cell was incubated in each increasing concentration of TEA Cl^- in neutral red for 20 min

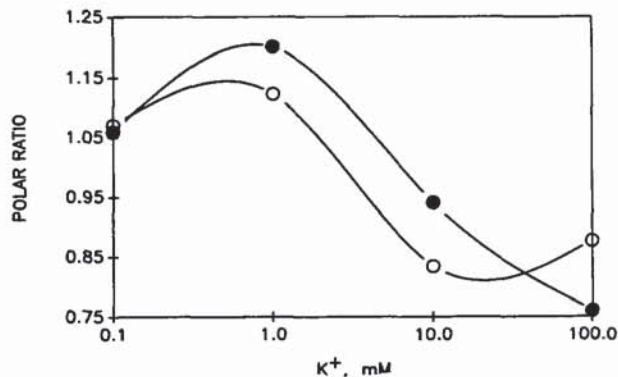


Fig. 9. The effect of KCl on the polar ratio. Each cell was sequentially treated with increasing concentrations of KCl. The cell was incubated in each concentration of KCl for 20 min. ○ Cell 1, ● cell 2

tons) of the protoplasm of a given cell can be varied according to the following equation:

$$B = (g) \text{Vol} (\rho_m - \rho_p) \quad (1)$$

where g is the acceleration due to gravity (9.8 m s^{-2}); Vol is the volume of the protoplast (in m^3) and ρ_m and ρ_p are the densities (in kg m^{-3}) of the external medium and protoplast, respectively. The density of the protoplast ρ_p of characean cells can be calculated from the densities of the endoplasm and the cell sap, which are $1.0145 \times 10^3 \text{ kg m}^{-3}$ and $1.0105 \times 10^3 \text{ kg m}^{-3}$, respectively (Kamiya and Kuroda 1957) and the relative volumes of the endoplasm and vacuole, which are 0.05 and 0.95, respectively. The calculated density of the protoplast is therefore $1.0107 \times 10^3 \text{ kg m}^{-3}$. When a cell ($40 \text{ mm} \times 0.6 \text{ mm}$) with a volume of $1.13 \times 10^{-8} \text{ m}^3$ is placed in APW (density $\approx 10^3 \text{ kg m}^{-3}$), its buoyancy is -1.18 μN . By contrast, the same cell's buoyancy is

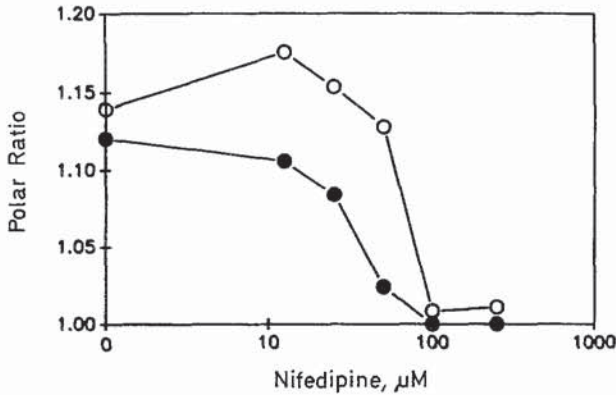


Fig. 10. The effect of nifedipine on the polar ratio. Each cell was incubated in increasing concentrations of nifedipine for 15 min. All treatments included 1% DMSO. \circ Cell 1, \bullet cell 2

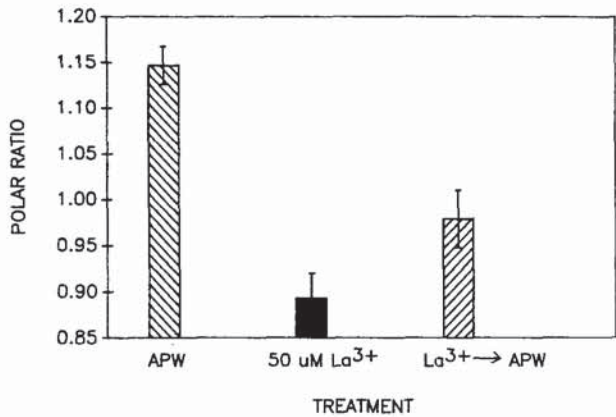


Fig. 11. The effect of LaCl_3 on the polar ratio. Cells ($n=3$) were treated with LaCl_3 for 20 min. In order to partially reverse the effect of La^{3+} the cells were washed with deionized water for approximately 6 h and placed in APW.

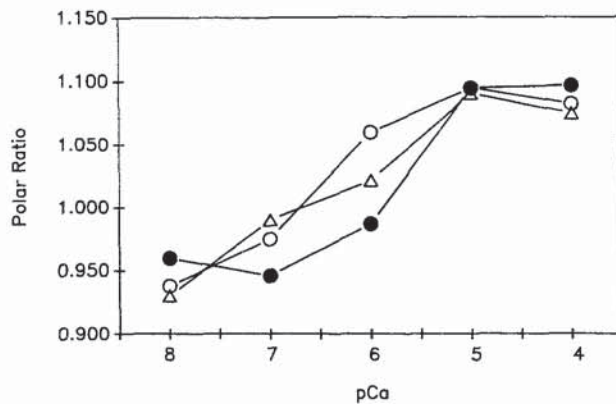


Fig. 12. The effect of external Ca^{2+} on the polar ratio. Cells were first treated with 1 mM EGTA (pH 7.2) for 5 min. Cells were then subjected to increasing free Ca^{2+} concentrations using EGTA buffers. The free Mg^{2+} concentration was kept constant at 0.1 mM. All treatments included 1 mM EGTA, 2 mM Hepes-NaOH (pH 7.2), 0.1 mM NaCl and 0.1 mM KCl. The coverslips were prewashed with EGTA. \circ Cell 1, \bullet cell 2, \triangle cell 3

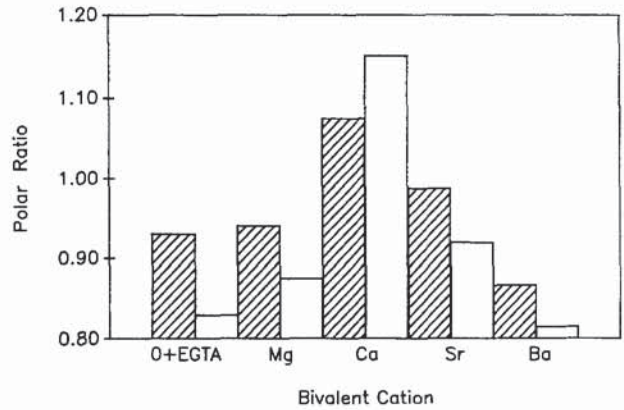


Fig. 13. The effect of bivalent cation on the gravity-induced polar ratio. Cells were first washed in 1 mM EGTA (pH 7.2) for 5 min. Each bivalent cation (0.1 mM) was added to 0.1 mM NaCl, 0.1 mM KCl and 2 mM Hepes-NaOH (pH 7.2). \square Cell 1, \boxtimes cell 2

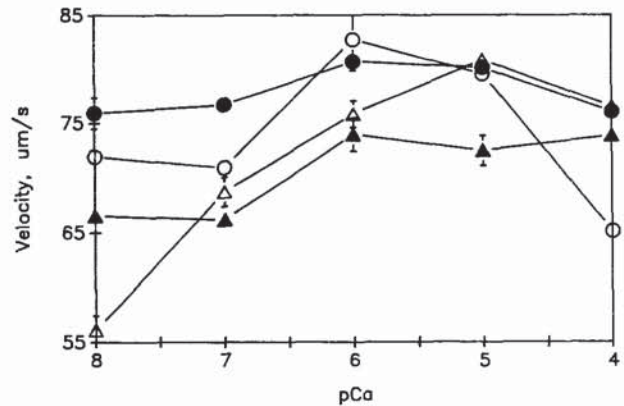


Fig. 14. The effect of external Ca^{2+} on the velocity of cytoplasmic streaming in horizontal cells. Cells were first treated with 1 mM EGTA (pH 7.2) for 5 min. Cells were then subjected to increasing free Ca^{2+} concentrations using EGTA buffers. The free Mg^{2+} concentration was kept constant at 0.1 mM. All treatments included 1 mM EGTA, 2 mM Hepes-NaOH (pH 7.2), 0.1 mM NaCl and 0.1 mM KCl. The coverslips were prewashed with EGTA. \circ Cell 1, \bullet cell 2, \triangle cell 3, \blacktriangle cell 4

2.88 μN when it is placed in APW containing 10% bovine serum albumin (density $\approx 1.03675 \times 10^3 \text{ kg m}^{-3}$). The whole protoplasm thus sinks in APW and floats in APW containing 10% BSA. Using this theoretical background, we tested whether or not the density of the external medium affects the polar ratio. Figure 5 shows that when the cells are placed in APW without BSA the polar ratio is greater than one. The polar ratio declines to one as the BSA concentration increases and eventually becomes less than one at high BSA concentration. The average BSA concentration that yields a polar ratio of 1.00 is $3.68\% \pm 0.98$ ($n=3$). This corresponds to a density of 1.0154, which is

slightly higher than the density of the protoplasm. The effect of BSA is completely reversible by washing the cell with APW (Data not shown).

The effect of the centrifugal acceleration on the polar ratio

Using the CMS-II centrifuge microscope of the stroboscopic type (Kamitsubo et al. 1989), we determined whether or not centrifugal acceleration is equivalent to gravitational acceleration. Figure 6 shows that centrifugal acceleration in the range from $4\text{--}30 \times g$ increases the streaming velocity of the bulk endoplasm in the centrifugal direction and decreases the streaming velocity in the centripetal direction. The polar ratio thus increases dramatically upon centrifugal acceleration. The effect of centrifugal acceleration is linear in the range in question (Each linear regression is very highly significant $P < 0.001$). After interpolating the curves to $1 \times g$, we can determine what the polar ratio is at a centrifugal acceleration of $1 \times g$. The polar ratio at $1 \times g$, determined in this manner is 1.11 ± 0.02 ($n = 6$). Centrifugal acceleration is thus qualitatively and quantitatively similar to gravitational acceleration in terms of its effects on the polarity of cytoplasmic streaming.

The centrifugal force needed to stop the cytoplasmic streaming of the bulk endoplasm in the centripetal direction (i.e. the balance acceleration) was also determined with the CMS-II centrifuge microscope. The average minimum and maximum balance accelerations are 148 ± 24 (16) $\times g$ and 178 ± 30 (16) $\times g$, respectively. Using the values found for the balance accelerations (α), we can estimate the motive force per unit area (F/A) responsible for driving cytoplasmic streaming in *Nitellopsis*. If we assume that the endoplasmic layer is 20×10^{-6} m in thickness (d) and the difference (D') in the densities of the cell sap and the endoplasm is 4 kg m^{-3} (Kamiya and Kuroda 1957), then the motive force per unit area is calculated to be between 0.12 and 0.14 N m^{-2} ($1.2\text{--}1.4 \text{ dynes/cm}^{-2}$) using the following formula:

$$F/A = d(D')\alpha g. \quad (2)$$

The effect of neutral red and cations on the polarity of cytoplasmic streaming

Serendipitously we found that staining the cell with neutral red results in a reversal of the gravity response. Cells stained with neutral red stream up faster than they stream down. This is reflected in the velocity profile (Data not shown). The effect of neutral red on the

polar ratio is concentration-dependent and reversible (Fig. 7). Since neutral red is known to activate a K^+ channel and cause a membrane hyperpolarization upon illumination (Kawamura and Tazawa 1980, Tazawa and Shimmen 1980), we tested the effect of the voltage-dependent K^+ channel blocker, tetraethylammonium Cl^- (TEA) on cells treated with neutral red and light. TEA prevents neutral red from reversing the gravity effect and consequently vertically positioned cells show a polar ratio greater than one whether the cells are given neutral red or not (Fig. 8). The effect of TEA is concentration-dependent. These data indicate that a hyperpolarization may be correlated with a reversed response to gravity. Perhaps this means that a depolarization may be correlated with a normal response to gravity. Indeed, treating the cells with 1 mM KCl , which depolarizes the membrane by approximately 59 mV , enhances the responsiveness of *Nitellopsis* to gravity (Fig. 9). Treatment of the cells with concentrations of KCl that will completely depolarize the membrane results in a reversed response to gravity.

Since a depolarization is often correlated with the opening of voltage-dependent Ca^{2+} -channels, we treated the cells with the voltage-dependent Ca^{2+} -channel blocker, nifedipine to determine whether or not Ca^{2+} influx through voltage-dependent channels is required for the graviresponse. Nifedipine inhibits the response in a concentration-dependent and reversible manner. Interestingly, nifedipine inhibition saturates at a polar ratio of one (Fig. 10). $LaCl_3$, a very potent, but irreversible inhibitor of the Ca^{2+} current in Characean cells, causes a reversed response to gravity (Fig. 11). The effect of $LaCl_3$ is only very weakly reversible by washing the cell with APW. Further experiments determined that Ca^{2+} contributes to the signal transduction chain involved in the coupling of the gravistimulation to the regulation of the polarity of this transport system.

Cells treated with Ca^{2+} -EGTA buffers reveal a Ca^{2+} -dependence in their response to gravity (Fig. 12). Cells treated with Ca^{2+} concentrations lower than $1 \mu\text{M}$ show a reversal in the normal response to gravity and thus have a polar ratio less than one. Cells treated with Ca^{2+} concentrations higher than $1 \mu\text{M}$ exhibit a normal response to gravity and a polar ratio greater than one. The response to gravity is supported half-maximally by $0.91 \mu\text{M } Ca^{2+}$. The polar ratio of horizontally placed cells is independent of external Ca^{2+} . The response to gravity requires Ca^{2+} specifically as a bivalent cation (Fig. 13). The order of effectiveness of other bivalent cations follows the sequence: $Ca^{2+} \gg Sr^{2+} > Mg^{2+} > Ba^{2+}$.

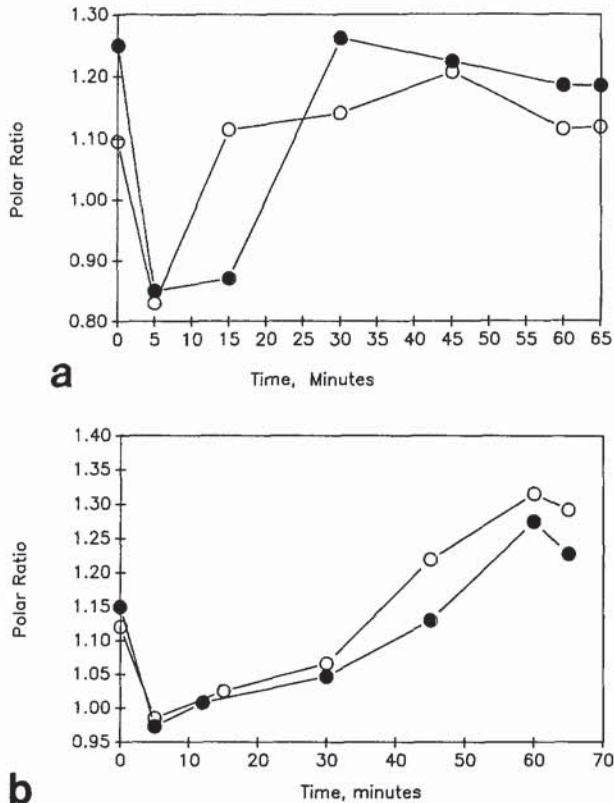


Fig. 15. Time courses of the effect of inhibitors of the Ca^{2+} -calmodulin complex on the polar ratio. **a** $2\ \mu\text{M}$ R 24571. R 24571 was dissolved in DMSO. All treatments included 0.01% DMSO. ○ Cell 1, ● cell 2, **b** $50\ \mu\text{M}$ W-7. W-7 was dissolved in APW (pH 7.2). ○ Cell 1, ● cell 2

Table 4. The effect of A-9-C on the polar ratio

Cell number	Polar ratio	
	APW	1 mM A-9-C
1	1.1839	1.1713
2	1.1436	1.1922
3	1.0912	1.0696
\bar{X}	1.1396	1.1444
S.D.	0.0470	0.0656
S.E.	0.0268	0.0378

Stock solutions of A-9-C (500 mM) were dissolved in 0.5 N NaOH. The pH of all the working solutions were maintained at 7.2 with 2 mM HEPES-NaOH. The cells were treated with or without A-9-C for 15 min before measurement

We find that the streaming velocity of intact horizontal cells is Ca^{2+} dependent (Fig. 14). The optimum concentration of Ca^{2+} is $1\ \mu\text{M}$; the threshold is $0.1\ \mu\text{M}$ and $0.4\ \mu\text{M}$ supports a half-maximal response on the

ascending portion of the curve and $12.5\ \mu\text{M}$ supports a half-maximal response on the descending portion of the curve. These data indicate that either lowering or raising the $[\text{Ca}^{2+}]$ around $0.4\ \mu\text{M}$ (the ascending portion) will slow down or speed up cytoplasmic streaming. (See Sato 1962 for similar experiments showing the effects of calcium on streaming in *Acetabularia*.) Gravity may induce a very slight increase in the intracellular $[\text{Ca}^{2+}]$ in the downwardly-directed stream and a very slight decrease in the $[\text{Ca}^{2+}]$ in the upwardly-directed stream. The ascending portion of the curve may be relevant to the gravity response whereas the descending portion of the curve is relevant to excitation-cessation coupling (Tominaga et al. 1983).

The effect of inhibitors of the Ca^{2+} -calmodulin complex on the polarity of cytoplasmic streaming

The Ca^{2+} concentrations required to activate the gravity response are in the range of physiological concentrations that activate calmodulin. In order to test the possibility that the Ca^{2+} -calmodulin complex participates in the gravity induced signal transduction chain, we treated the cells with the Ca^{2+} -calmodulin complex inhibitors, W-7 ($50\ \mu\text{M}$) and R 24571 ($2\ \mu\text{M}$). Both drugs show complex inhibition kinetics (Fig. 15). Both drugs cause a reversal of the polarity within 5 minutes after treatment. This is followed by a return to the normal polarity within 15 minutes and then an enhanced polarity. The degree of enhancement depends upon the drug concentration (Data not shown). The effect of these inhibitors may be non-specific since $50\ \mu\text{M}$ W-5, a less potent analogue of W-7, yields similar results. The reversal of polarity can not be obtained again by retreating the cells with fresh inhibitor.

The effect of an inhibitor of the Cl^- channel on the polarity of cytoplasmic streaming

Since Ca^{2+} activates the Cl^- channel in *Nitellopsis*, we tested whether the activation of the Cl^- channel is required for the graviresponse. The opening of the Cl^- channel results in the inward current observed during the action potential. Treating the cells with 1 mM A-9-C, an inhibitor of the Cl^- channel in *Nitellopsis* (Shiina and Tazawa 1987a, 1988), does not have any detectable effect on the graviresponse (Table 4).

Discussion

The effect of gravity on the polarity of cytoplasmic streaming

Cells of *Nitellopsis obtusa* show a polarity in the velocity of cytoplasmic streaming that is dependent on gravity.

The velocity of the downwardly flowing endoplasm is approximately 11 percent faster than the velocity of the upwardly flowing endoplasm. We define the velocity of the downwardly directed stream divided by the velocity of the upwardly directed stream as the polar ratio. Gravity also influences the polar ratio in *Chara braunii* (Hayashi 1957), *Nitella*, *Elodea* and *Vallisneria* (Ewart 1903) as well as in *Avena* (Bottelier 1934). Our observations show that the polar ratio of vertically placed cells of *Nitellopsis* is 1.11. Ewart (1903) found that the polar ratios of vertically positioned cells of *Nitella*, *Elodea* and *Vallisneria* are 1.10, 1.11, and 1.04, respectively. Hayashi (1957) found that the polar ratio of *Chara braunii* is 1.04. Bottelier (1934) found that the polar ratio of *Avena* is 1.29. The polar ratio of vertically-oriented *Chara* rhizoids is approximately 1.04 if we subtract the effects of the endogenous polarity (Hejnowicz et al. 1985). It appears that the influence of gravity on the polarity of streaming is common. Indeed, gravity may influence cytoplasmic streaming in all streaming cells.

The internodal cells of *Nitellopsis* respond to gravity without any detectable lag time and the magnitude of the response depends on the cosine of the azimuth of the actin bundles relative to gravity (horizontal orientation = 0°). Although there is a gravity-induced polarity in the velocity of cytoplasmic streaming in vertically oriented cells, there is not any accumulation of cytoplasm on the bottom on the cell. This is a consequence of the fact that the thickness of the retarded upwardly-directed stream is greater than the thickness of the accelerated downwardly-directed stream. Even in cells whose polarity of cytoplasmic streaming has been reversed by NR, a buildup of cytoplasm does not occur. In NR treated cells, the retarded downwardly-directed stream is thicker than the accelerated upwardly-directed stream. The gravity-induced polarity of cytoplasmic streaming follows the law of continuity of fluids (Vogel 1983).

The effects of neutral red on gravity-induced polarity of cytoplasmic streaming

The polar ratio is reversed when the cells are placed in NR. Although NR is usually considered to be only a nonintrusive dye that enhances contrast as a result of its pH-dependent partitioning into cellular compartments, NR also has pharmacological effects. For instance, Kawamura and Tazawa (1980) find that NR mediates a rapid light-dependent hyperpolarization of the plasma membrane in the cells of *Chara*. Although

the molecular action of NR remains unknown in *Nitellopsis*; in *Chara*, it permits the opening of a K⁺ channel following the absorption of light by the chloroplasts. Furthermore NR accumulates in a pH-independent manner in the tannin vesicles of *Mougeotia* and subsequently inhibits chloroplasts movement (Russ et al. 1988, Grolig and Wagner 1989). Russ et al. (1988) hypothesize that the inhibition results from a blockage of the light-induced Ca²⁺ efflux from the vesicles. It is clear that NR affects the ionic relations of plant cells.

The effect of ions on gravity-induced polarity of cytoplasmic streaming

In order to test the possibility that NR influences the polar ratio by activating a K⁺ channel, we further treated NR-processed cells with TEA Cl⁻. The observation that TEA Cl⁻ nullifies the effect of NR supports the contention that NR mediates the opening of a K⁺ channel and consequently induces a membrane hyperpolarization. These observations stimulated experiments to determine whether or not electrical and/or ionic mediators are integral components of the gravity-dependent regulation of the polarity of cytoplasmic streaming.

External Ca²⁺ is required for the expression of the graviresponse in *Nitellopsis*. Perhaps this is expected since Ca²⁺ is also involved in various steps leading to gravitropism in higher plants. Ca²⁺ participates in the phytochrome-mediated induction of the graviperception machinery in Merit corn (Perdue et al. 1988), in the electrical changes that occur minutes after gravistimulation (Björkman and Leopold 1988), and in the final bending response (Lee et al. 1983a, b; Moore 1985b, c; Slocum and Roux 1983). In *Nitellopsis*, however, the direction of the graviresponse depends on the external Ca²⁺ concentration; concentrations greater than 1 μM yield a normal response, concentrations less than 1 μM lead to a reversed response. Therefore the quality as well as the quantity of the response is affected by Ca²⁺. This response is reminiscent of the effect of Ca²⁺ on the motility of cilia (Kamiya and Witman 1984).

One particularly interesting and novel observation is that the inhibition of the gravity response by nifedipine saturates at a polar ratio near 1.00. This indicates to us that *Nitellopsis* has at least two kinds of Ca²⁺ channels; one that is inhibited by nifedipine and one that is not. These channels may be homologous with the

nifedipine-sensitive L-type channels and the nifedipine-resistant N-type channels in animal cells (Tsien et al. 1987). This interpretation is supported by the observation that La^{3+} , which completely inhibits the Ca^{2+} current in Characean cells (Shiina and Tazawa, 1987b, Tsutsui et al. 1987), reverses the normal gravity-induced polarity in *Nitellopsis*. It thus appears that both types of channels are involved in determining the polarity of cytoplasmic streaming. MacRobbie and Banfield (1988) conclude from $^{45}\text{Ca}^{2+}$ experiments that two kinds of Ca^{2+} channels exist in *Chara*; both open upon depolarization but only one is sensitive to nifedipine. It is likely that a gravity induced membrane depolarization open both types of Ca^{2+} channels in *Nitellopsis*. Once Ca^{2+} enters the cell it is likely that it will bind to calcium binding proteins, including calmodulin. Calmodulin has been detected in Characean cells (Tomimaga et al. 1985) and may participate in the graviresponse of *Nitellopsis*. The Ca^{2+} -calmodulin antagonists, R 24571 and W-7 transiently reverse the direction of the response to gravity. One explanation for these kinetics derives from the possibility that calmodulin activates two opposing reactions that are spatially separated or differentially sensitive to the antagonists. A calmodulin-activated protein kinase and a calmodulin-activated protein phosphatase are possible candidates for the receptor proteins that may influence the polarity of cytoplasmic streaming in *Nitellopsis*. There is also a possibility that the effect of the inhibitors is nonspecific. For example, the uptake of the calmodulin antagonists may cause a transient depolarization of the membrane that results in the observed response. Potassium ions also have the ability to regulate the polar ratio. Slightly depolarizing the membrane with 1 mM KCl sensitizes the cell to gravity. Low concentrations of K^{+} may influence the graviresponse by facilitating the opening of voltage-dependent Ca^{2+} channels. The effects of K^{+} are more complicated since higher concentrations lead to a reversed response to gravity. It is possible, however, that treating the cells with 10–100 mM KCl limits the transplasmalemmal movement of Ca^{2+} by lowering the electrochemical gradient that drives the movement of Ca^{2+} into the cell. This would be a reasonable interpretation if gravity affects the membrane potential, which in turn influences the influx of Ca^{2+} which then influences the polarity of streaming. We have not eliminated the possibility that the membrane potential itself influences the polarity of streaming, and the external Ca^{2+} concentration influences the membrane potential.

Localizaton of the site of graviperception

Němec (cited in Haberlandt 1914) identified the site of graviperception in roots through amputation experiments. He found that when he removed the rootcap containing statocysts, the cells were unable to respond to gravity, indicating that the rootcap is the site of perception. Haberlandt (1914) carried out analogous experiments on the shoots of *Tradescantia virginica*. He found that the basal region of each internode is needed to perceive gravity. Using cellular ligation, we have also performed amputation experiments in order to identify regions of the cell that may serve as the site of graviperception.

The relationship between the external calcium ion concentration and the gravity-induced polarity of cytoplasmic streaming indicates that the gravireceptor and/or early elements in the signal transduction chain may reside on or near the plasma membrane. This is further supported by the inhibition of the gravity-induced polarity by nifedipine and La^{3+} . The inability of singly or doubly ligated cells to respond to gravity suggests that the membrane region adjacent to the nodes may be specialized for graviperception and/or signal transduction. Of particular interest is the fact that the plasmodesmata are localized in this region. Is it possible that the plasmodesmata participate in realizing the gravity-induced polarity of cytoplasmic streaming? The plasmodesmata on the top of the internodal cell are subjected to a different pressure differential than the plasmodesmata on the bottom of the internodal cell. Ding and Tazawa (1989) have shown that the conductance of the plasmodesmata of *Chara* is modified by the difference in the hydrostatic pressure across the plasmodesmata. Could a gravity induced pressure differential result in the differential gating of the plasmodesmata and the subsequent induction of a polarity in cytoplasmic streaming by gravity? The effect of gravity on transport through plasmodesmata remains a neglected area of research.

Could gravity act only upon the streaming endoplasm, an element in the final step of the graviresponse? It is possible to determine whether or not gravity is working exclusively on the endoplasm. In order to accomplish this task, we must determine the increment or decrement of the velocity relative to the horizontal velocity. From these data we can calculate the centrifugal acceleration that is needed to stop streaming in the centripetal direction (Hayashi 1957). Using the data from Table 1, we find that the average rates of upwardly-directed and downwardly-directed streaming are

94.8 $\mu\text{m/s}$ and 102.6 $\mu\text{m/s}$, respectively for upright and inverted cells. If the endogenous rate of streaming is called U and the rate of streaming influenced by gravity is termed V , then

$$U - V = 94.8 \quad (3)$$

$$U + V = 102.6 \quad (4)$$

Therefore the rate of streaming that results from the endogenous motive force is 98.7 $\mu\text{m/s}$ and the increment or decrement in the rate of streaming that is a consequence of gravity is 3.9 $\mu\text{m/s}$. According to Hayashi (1957), the ratio, U/V , which equals 25.3 should predict the amount of centrifugal acceleration needed to stop streaming in the centripetal direction. The balance acceleration for the bulk endoplasm, determined empirically with the use of a centrifuge microscope is between 148 and $178 \times g$ in *Nitellopsis*. The balance acceleration is $40 \times g$ for *Chara* (Hayashi 1957) and greater than $50\text{--}100 \times g$ (Kamitsubo et al. 1989) and between 200 and $800 \times g$ (Kamiya and Kuroda 1958) for the internodal cells of *Nitella*. Most of these values are much higher than the predicted balance acceleration. The underlying assumption of Hayashi's prediction is that gravity only acts upon the endoplasm and the effect of gravity is linear. We show that the effect of centrifugal acceleration is linear in the range in question. It is therefore possible that gravity acts not only on the endoplasm, but also elsewhere, for instance on a plasma membrane receptor where the effect of gravity is amplified. Since the balance acceleration is substantially greater than 25.3 we assume that the endoplasm is not the only gravireceptor.

Since we find that both ends of the cell are necessary for graviperception it is reasonable to ask how the two ends communicate. The lag time of the response is too fast for chemical communication through the streaming endoplasm. Furthermore, a communication between the two ends by means of an action potential has been ruled out by the lack of effect of A-9-C on graviperception. A-9-C blocks the Cl^- channel, which carries the inward current during an action potential and thus inhibits the action potential. At present, we think that it is likely that the two ends communicate through a difference in the membrane potential at the top and bottom of the cell.

A new model for graviperception

It is generally assumed that the plasma membrane does not act as the gravireceptor because it lacks the sensitivity to directly sense the gravitational force. How-

ever, mechanosensitive channels are now becoming well characterized (Edwards and Pickard 1987, Roberts et al. 1988). Indeed, energies as low as 8×10^{-20} J will open a single cation channel in hair cells (Howard et al. 1988). This energy is 5.9×10^4 times smaller than the potential energy due to the weight of the protoplasm itself and it is in the neighborhood of the energy of thermal noise. If, in fact, the gravireceptors are localized in the two cell ends, and a differential force is required in order to sense gravity, then the detection system is protected against being nonspecifically activated by thermal noise or changes in cell turgor. It is possible that the differential force exerted on the top and bottom membrane, by the pulling away and falling of the cytoplasm, respectively is enough to activate a gravireceptor.

Czapek (1898) and Pickard and Thimann (1966) proposed that graviperception resulted from the weight of the protoplasm upon the lower membrane. Audus (1971), although considering the "protoplast weight theory" unlikely, suggested that a "pull from below" is just as likely as a "push from above". In light of the observations that both cell ends are required for graviperception in *Nitellopsis*, it is possible that both mechanisms are operating in the gravity-dependent polarity of cytoplasmic streaming in *Nitellopsis*.

Edwards and Pickard (1987) have elaborated on the above model by suggesting that the cytoskeleton may funnel the small amount of energy that results from the gravity-induced pressure on the plasma membrane directly to stretch-activated channels. While our data indicate that there is a relationship between the plasma membrane and the motile machinery, it neither supports nor refutes their model.

The experiments designed to change the static buoyancy show that *Nitellopsis* cells are capable of sensing the weight of the protoplast. The potential energy inherent in a *Nitellopsis* protoplasm is 4.72×10^{-6} J for every meter it falls or 4.72×10^{-15} J if it falls only 1 nm (molecular dimensions). This is still one million times greater than the energy in thermal noise (10^{-21} J). That is, the signal-to-noise ratio is 4.72×10^6 . The cells of *Nitellopsis*, however are approximately one million times larger than a typical ($20 \times 10^{-6} \text{ m}^3$) higher plant cell. Could the plasma membranes of a small higher plant cell also detect the weight of the protoplasm? Perhaps – if the density of the small cells could be increased. The presence of starch-filled amyloplasts may fulfill this function. That is, starch grains would not have to fall, as the classical theory dictates; they would just have to be present in the statocytes in order to

increase the density of the protoplasm. According to our hypothesis, the starch grains would function simply as ballast.

We can estimate the amount of energy released from a ($20 \times 10^{-6} \text{ m}^3$) columella protoplast as it falls $1 \times 10^{-9} \text{ m}$ using the following formula:

$$\text{Potential Energy} = (\text{Static Buoyancy})(\text{Distance}) \quad (5)$$

where

Static Buoyancy

$$= \text{Vol} [\rho_m - (\tau_v \rho_v + \tau_a \rho_a + \tau_c \rho_c)] g \quad (6)$$

where Vol equals the total volume of the protoplast, g represents the acceleration due to gravity, τ_v , τ_a , and τ_c represent the relative volumes of the vacuole, amyloplasts and cytoplasm, respectively and ρ_m , ρ_v , ρ_a , and ρ_c represent the densities of the medium, vacuole, amyloplasts and cytoplasm, respectively.

If the vacuole, amyloplasts and remaining cytoplasm occupy 13.8, 10.5, and 75.5 percent of the protoplasm, respectively (Moore and Pasieniuk 1984, Ransom and Moore 1984, Moore 1985a), and the densities of these three components are 1.0105×10^3 , 1.5×10^3 , and $1.0145 \times 10^3 \text{ kg m}^{-3}$, respectively; then the static buoyancy of this cell is $-5.1 \times 10^{-12} \text{ N}$. The potential energy released from this cell falling $1 \times 10^{-9} \text{ m}$ is $5.1 \times 10^{-21} \text{ J}$: approximately the energy needed to open one ion channel. Is the activation of one ion channel enough to provide biochemical information to the cell? Since micromolar concentrations of calcium ions are required for orthogravitropism in corn (Perdue, Leopold and Wayne in preparation), let us consider the possibility that the falling of the protoplasm opens a calcium channel. If we assume that one ion channel can pass 10^6 ions s^{-1} , it will take approximately 3s to raise the calcium concentration of the above cell from $1 \times 10^{-7} \text{ M}$ to $1 \times 10^{-6} \text{ M}$. These calculations show that the proposed function of statoliths of higher plants is reasonable.

Using a similar argument we can see that a falling *Nitellopsis* protoplast that has a volume of $45.2 \times 10^{-9} \text{ m}^3$ has enough energy to activate 5.9×10^4 ion channels. This is sufficient to raise the intracellular calcium concentration from $1 \times 10^{-7} \text{ M}$ to $1 \times 10^{-6} \text{ M}$ in approximately 0.4s. More research is needed to determine whether or not calcium channels are being activated by the compression and/or tension on the membrane. We only present these calculations to show that both higher plant columella protoplasts and *Nitellopsis* internodal protoplasts contain similar potential energies in a gravitational field; energies that are sufficient

to cause an increase in the concentration of at least one biologically significant messenger.

The greater the density of the entire protoplast, the greater the signal to noise ratio will be in detecting gravity. This may explain why starch-less roots of *Arabidopsis* still detect gravity, although with a much greater variability. The observations in *Arabidopsis* may be a consequence of the presence of a gravireceptor in both the wild type and starch-less mutant, but a lower signal to noise ratio in the mutant relative to the wild type (Caspar and Pickard 1989, Kiss et al. 1989). Further we can estimate that the a starchless protoplast will have approximately 23% of the weight of a starch-containing protoplast by substituting 1.0145×10^3 for $1.5 \times 10^3 \text{ kg m}^{-3}$ in Eq. 6. Therefore the starchless protoplasts will have 23% of the potential energy of the starch containing-protoplasts in a gravitational field. The results of Kiss et al.(1989) show that the presentation time required by roots which have starch containing columella cells to perceive gravity is 0.4 minutes; whereas the presentation time of the roots containing starchless columella cells is between 1.3 and 1.9 minutes. This means that the starchless columella cells are between 21% and 31% as efficient in detecting gravity as the starch containing columella cells. This is just what we predict if starch is only acting as a material to increase the density of the protoplast *in toto*.

The function of statoliths proposed above is for higher plants only and is not meant to explain the action of the statoliths found in the rhizoids of *Chara*. In this case it seems clear that the falling of the heavy BaSO_4 crystals displace the dictyosomes so that wall precursors can only be delivered to the upper side and consequently the rhizoid grows down (Sievers and Volkman 1979).

How may gravity-induced polarity in cytoplasmic streaming regulate other graviresponses?

While we consider the gravity-induced polarity in cytoplasmic streaming to be a graviresponse in its own right, it is possible that it may act as a component of signal transmission in other graviresponses, including gravitropism. When diffusion becomes limiting, cytoplasmic streaming becomes a very efficient method for the rapid dissemination and/or mixing of metabolites in plant cells (Ding and Tazawa 1989, Lucas and Shimmen 1981). According to Fick's Law combined with the Einstein-Stokes Equation (Dörr 1983), the flux of a spherical molecule (dn/dt , in number of particles per

second) depends upon the temperature (T , in Kelvin), the radius of the molecule (a), the viscosity of the solvent (η , in Pascals-seconds) for a specific molecule (Luby-Phelps et al. 1988) and the density gradient of the particles over a distance x (dc/dx , in units of moles m^{-3}/m).

$$dn/dt = \frac{-(kT)}{6\pi\eta a} A dc/dx \quad (7)$$

where k is Boltzmann's constant ($1.38 \times 10^{-23} \text{ JK}^{-1}$); π equals 3.14 and A is the area where the particles cross during time, t . The diffusion coefficient (D) is equal to $kT/6\pi\eta a$. Therefore diffusion becomes limiting and cytoplasmic streaming becomes important for the delivery of metabolites when the concentration gradient is small, the cytoplasmic viscosity is high and/or the molecular radius is large. The viscosity of the bulk endoplasm of *Nitella axilliformis* that is experienced by lipid droplets is approximately 1 Pa s (Kamitsubo et al. 1988). This is 1,000 times the viscosity of water and may severely limit diffusion. If cytoplasmic streaming is responsible for the delivery of an essential substance, substances, or organelles that control various gravi-responses then a gravity-induced polarity in cytoplasmic streaming can provide a mechanism for the non-random distribution of these substances or organelles and consequently an amplification of the gravistimulus. Indeed, if such an essential substance whose distribution is limited by diffusion can regulate growth, then a gravity-induced polarity in cytoplasmic streaming may participate in the regulation of gravitropism (Audus 1979, Kessler 1979).

It is also possible that the gravity-induced differential in the upward and downward streaming velocities may affect gravitropism by having a direct effect on membrane permeability. This is possible if shear stress-activated ion channels exist in plants-particularly in cells that have rotational streaming. Bovine aortic endothelial cells have a K^+ channel that is half-maximally activated in a sigmoidal manner by a shear stress of 0.07 N m^{-2} (Olesen et al. 1988). The motive force per unit area (i.e. shear stress) that drives cytoplasmic streaming in *Nitellopsis* and *Nitella*, for example, are approximately 0.13 N m^{-2} and 0.16 N m^{-2} , respectively. Therefore it is possible that the differential velocities of the upward and downward streams may influence the ionic permeability of their adjacent membranes to varying extents.

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