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THE EFFECT OF THE EXTERNAL MEDIUM ON THE GRAVITY-INDUCED POLARITY OF CYTOPLASMIC STREAMING IN *CHARA CORALLINA (CHARACEAE)*[1]

MARK P. STAVES,[2,4] RANDY WAYNE,[2] AND A. CARL LEOPOLD[3]

[2]Section of Plant Biology, Cornell University, Ithaca, New York 14853; and
[3]Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, New York 14853

Gravity induces a polarity of cytoplasmic streaming in vertical internodal cells of *Chara* such that the downwardly directed stream moves faster than the upwardly directed stream. In order to determine whether the statolith theory (in which intracellular sedimenting particles are responsible for gravity sensing) or the gravitational pressure theory (in which the entire protoplast acts as the gravity sensor) best explain the gravity response in *Chara* internodal cells, we controlled the physical properties of the external medium, including density and osmolarity, with impermeant solutes and examined the effect on the polarity of cytoplasmic streaming. As the density of the external medium is increased, the polarity of cytoplasmic streaming decreases and finally disappears when the density of the external medium is equal to that of the cell (1015 kg/m^3). A further increase in the density of the external medium causes a reversal of the gravity response. These results are consistent with the gravitational pressure theory of gravity sensing since the buoyancy of the protoplast is dependent on the difference between the density of the protoplast and the external medium, and are inconsistent with the statolith theory since the buoyancy of intracellular particles are unaffected by changes in the external medium.

Key words: *Chara corallina*; Characeae; cytoplasmic streaming; density; gravitational pressure; gravity sensing; osmolarity.

Gravity is unique among environmental stimuli in that it is ever-present and has a constant value. The incessant action of gravity makes it an excellent candidate for a growth-regulating signal and, indeed gravity influences many developmental events, most notably the polarity of the root/shoot axis of higher plants and the orientation of animals (Wayne and Staves, 1996). How is this influential gravity signal perceived? In answering this question, roots have been popular experimental systems since the gravitropic curvature is easily observed. However in these multicellular organs, various cells are involved in the processes of gravity sensing, transmission of a signal from the root cap, and differential growth; and presently, the sensing cells of higher plants cannot be studied comprehensively and unambiguously in isolation. Thus, the interpretation of experiments designed to study plant gravisensing with these organs is complicated. Many of the complexities may be obviated by the use of single cells that exhibit a gravity response. Studying gravity responses in single cells ensures that perception and response occur within the cell being observed, and that the sensing cells can be directly manipulated. The single internodal cells of the characean algae exhibit a gravity-induced polarity of cytoplasmic streaming and provide just such advantages for studying gravisensing in plants.

The large internodal cells of the characean algae exhibit a highly organized and rapid rotational cytoplasmic streaming (Corti, 1774). The motive force for streaming is generated by the movement of myosin along actin cables, which are located at the ectoplasm/endoplasm interface just interior to the layer of chloroplasts embedded in the ectoplasm (Kamiya, 1959). In horizontal internodal cells of *Chara corallina*, the cytoplasm streams right and left at ~100 μm/s. In vertical cells, however, there is a polarity of cytoplasmic streaming such that the downwardly directed stream moves ~10% faster than the upwardly directed stream (Staves, Wayne, and Leopold, 1992, 1995). We refer to the velocity of the downstream divided by the velocity of the upstream as the polar ratio (Wayne, Staves, and Leopold, 1990). Thus a vertical internodal cell normally has a polar ratio of ~1.10. Gravity-induced polar ratios also occur in other characean algae (Ewart, 1903; Hayashi, 1957; Hejnowicz, Buchen, and Sievers, 1985; Wayne, Staves, and Leopold, 1990; Buchen et al., 1991) and in higher plants (Ewart, 1903; Bottelier, 1934). The polarity of cytoplasmic streaming is under physiological control and is not a result of the direct influence of gravity on individual particles in the cytoplasm (Wayne, Staves, and Leopold, 1990, 1992; Staves, Wayne, and Leopold, 1992, 1995).

We have used the polarity of cytoplasmic streaming in characean internodal cells as a rapid, noninvasive assay for gravity sensing at the cellular level. As an outcome of this study we have offered a novel theory, the gravitational pressure theory, to explain gravisensing in algal, higher plant, and animal cells. In this theory, gravity acts on the entire protoplast causing it to settle within the confines of the cell wall or extracellular matrix (ECM). We envision that this settling causes a tension between the plasma membrane and the ECM at the top of the cell and a compression between the plasma membrane and the ECM and the bottom of the cell. The differential tensions and compressions caused by the gravity-induced settling of protoplasts in internodal cells are actually

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[4] Current address, author for correspondence: Department of Biology, Grand Valley State University, Allendale, MI 49401. [Tel: (616) 895-2473, FAX: (616) 895-3412, email: stavesm@gvsu.edu].

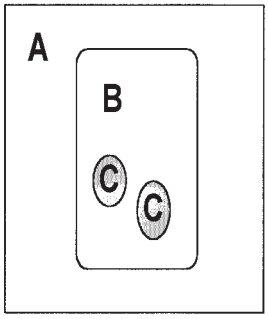
small reductions of pressure due to turgor at the tops of the cells, and augmentations of pressure due to turgor at the bottoms of cells. The differential pressures activate the gravireceptor(s), which reside at the plasma membrane-ECM junction. This activation by gravitational pressure can be mimicked by unidirectionally applied hydrostatic pressure (Staves, Wayne, and Leopold, 1992). We suggest that the most likely candidates for the gravireceptor(s) are integrin-like proteins, which span the plasma membrane–ECM junction and are localized at the ends of the cells (Wayne, Staves, and Leopold, 1992).

We proposed the gravitational pressure theory (Wayne, Staves, and Leopold, 1990) as an alternative to the statolith theory in which intracellular particles are thought to act as the gravity sensor either by sedimenting within the cell (Sack, 1991) or by exerting a force on components of the cytoskeleton (Sievers et al., 1991).

The gravitational pressure theory predicts that the polar ratio will diminish as the density of the external medium increases. When the density of the external medium approaches that of the protoplast, the protoplast will become neutrally buoyant; thus, it will no longer settle within the ECM and there will be no gravity response (polar ratio = 1.0). Increasing the density of the external medium beyond this point will cause the protoplast to become buoyant within the ECM, which will create a compression between the plasma membrane and the ECM at the top of the cell and a tension between the plasma membrane and the ECM at the bottom of the cell. Since this is the inverse of the normal condition, it will result in a reversal of the gravity response, i.e., the velocity of the upwardly directed stream will be greater than that of the downwardly directed stream (polar ratio $<$ 1.0).

By contrast, the statolith theory would predict that varying the density of the external medium will have no effect on gravity sensing. This is because, according to Pascal (1663), Boyle (1690), and the Principle of Archimedes, the buoyancy of intracellular particles is solely a function of the difference between the density of the particle and the density of the surrounding cytoplasm, the volume of the particle, and the acceleration due to gravity (Fig. 1). Thus, changes in the extracellular medium will affect the buoyancy of the whole protoplast, but will not affect the buoyancy of intracellular particles.

In practice, the proposed experiment to test the two theories is not so straightforward. While the density of a solution can be increased by increasing the concentration of a solute dissolved in it, the addition of a solute to a medium will also increase both the density and the osmolarity of the solution. Since the osmolarity of the external medium also influences the gravity-induced polarity of cytoplasmic streaming (Staves, Wayne, and Leopold, 1992), we performed experiments designed to separate the contribution of osmolarity and density to the gravity response in *Chara* internodal cells. To accomplish this, we measured the polar ratios of the cells in solutions of chemically diverse molecules that differentially alter the osmolarity and density of the external medium. Here we present data that demonstrate that the density of the external medium affects the ability of *Chara* internodal cells to sense gravity. These data are consistent with the gravitational pressure theory, but not with the statolith theory of gravisensing.



$$\text{BUOYANCY}_C = \underline{V}_C(\rho_B - \rho_C)\underline{g}$$

$$\text{BUOYANCY}_B = \underline{V}_B\left\{\rho_A - \left[\left(\frac{\underline{V}_C}{\underline{V}_C + \underline{V}_B}\right)\rho_C + \left(\frac{\underline{V}_B}{\underline{V}_C + \underline{V}_B}\right)\rho_B\right]\right\}\underline{g}$$

Fig. 1. The relationship between the densities of the external medium (A), the protoplast (B), and intracellular particles (C) on the buoyancy of the protoplast and/or the particles, where V = volume, ρ = density, and g = gravitational acceleration. When solution (A) is not in immediate communication with a structure, for example, the intracellular particles (C), that solution has no effect on the velocity, force, or energy available to do work from the falling of (C). In order to visualize the physical situation, imagine that the intracellular particles are stones inside a water-filled balloon. The stones fall whether the balloon is immersed in air, water alone, or water plus any solute that is impermeant to the balloon membrane. By contrast, the buoyancy of the water-filled balloon containing the stone will depend on the density of the external medium (A).

MATERIALS AND METHODS

Chara corallina Klein ex Wild., em. R.D.W.(= *Chara australis* R. Brown) was cultured in aquaria containing soil water medium as described in Wayne and Staves (1991). Freshly isolated, apical internodal cells of *Chara* (2–3 cm long) were mounted in a glass/leucite chamber containing a solution of the molecule of interest dissolved in artificial pond water (APW; 0.1 mmol/L NaCl, 0.1 mmol/L KCl, 0.1 mmol/L $CaCl_2$, osmolarity $\approx$ 0.6 Osm/m^3 (1.49 kPa), density $\approx$ 1000 kg/m^3). The chamber was placed on the stage of a horizontally oriented Olympus CH-2 microscope equipped with a 20$\times$ (A20PL, NA [numerical aperture] = 0.4; or SPLAN20, NA = 0.46) objective lens and 10$\times$/18L CWHK oculars. The cells were treated sequentially with increasing concentrations of a given solute and were allowed to equilibrate at each new concentration for 20 min. Longer incubations (up to 180 min) resulted in no additional effect of the solute on the speed or polarity of cytoplasmic streaming (data not shown). Cytoplasmic streaming was measured with the chloroplasts in focus in order to sample the streaming rate at the site of the generation of the motive force (Staves, Wayne, and Leopold, 1995). The streaming velocity was determined by measuring the time required for cytoplasmic particles to travel 250 μm. Twenty-five measurements were made in each direction and the polar ratio was determined for vertically oriented cells by calculating the ratio of the velocity of the downwardly directed stream to that of the upwardly directed stream.

The osmolarities of all solutions were determined with a freezing-point depression osmometer (Fiske and Associates, model one-ten, Needham, MA) and densities were measured gravimetrically with 1-mL samples on a Sartorius (model R160-P, Edgewood, NY) analytical balance. For each solution, the relationship between osmolarity and density was determined from a first-order regression (Fig. 2).

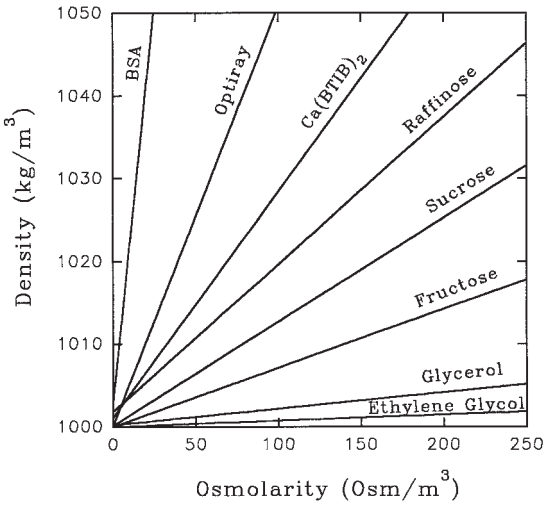


Fig. 2. First-order regressions, showing the relationship between osmolarity and density for (A) bovine serum albumin (R = 0.9844); (B) Optiray (R = 0.9997); (C) $Ca(BTIB)_2$ (R = 0.9999); (D) raffinose (R = 0.9931); (E) sucrose (R = 0.9997); (F) fructose (R = 0.9999); (G) glycerol (R = 0.9993); and (H) ethylene glycol (R = 0.9998). Regressions were drawn through each of the 5–7 points measured for a given solute. Osmolarity, or osmotic concentration (c_s, in Osmol/m^3), may be converted to osmotic pressure (π_s, in Pascals) by using the following relation: $\pi_s = RTc_s$; where R is the gas constant (8.31 Pa · m^{-3} · mol^{-1} · K^{-1}) and T is the temperature in Kelvin (see Discussion).

To remove the contents of *Chara* internodal cells, one end of an internodal cell was removed with a scissors and the cell contents were expressed with a spatula onto a piece of Parafilm (American Can Company, Chicago, IL) leaving behind a clear, empty cell wall. The osmolarity of the entire protoplast was determined with a freezing-point depression osmometer (Fiske and Associates, model one-ten, Needham, MA). Densities of 5-μL samples, obtained with a Drummond (model 210, Bromall, PA) microdispenser, of the protoplast (of two cells) were measured gravimetrically using a Sartorius (model R160-P) analytical balance. Each microdispenser tube was calibrated with distilled water using the relation:

$$\frac{\text{mass of protoplast in tube}}{\text{mass of water in tube}} = \frac{\text{density of protoplast}}{\text{density of water}}$$

where the density of water was taken to be 1000 kg/m^3 at room temperature.

Chemicals were purchased from Sigma (St. Louis, MO), with the exception of Optiray, which was a gift from Mallinkrodt (St. Louis, MO). The iodinated compound [$Ca(BTIB)_2$] was prepared by dissolving 3,5-bis[acetylamino]-2,4,6-triiodobenzoic acid with $Ca(OH)_2$ to form a calcium salt with a molecular mass of ~1266 Da.

Statistical analyses were performed using Minitab (Minitab Inc., University Park, PA) and SigmaPlot (Jandel Scientific, San Rafael, CA). All data are presented as the mean ± the standard error of the mean.

RESULTS

We find that increasing concentrations of all the solutes tested causes a decrease in the ability of cells to sense gravity as analyzed by the polar ratio (Fig. 3). Solutes with molecular masses <200 g/mol decrease the polar ratio asymptotically to ~1.0, whereas solutes of higher molecular mass cause the polar ratio to fall below 1.0.

In order to separate the effects of density and osmolarity on gravisensing, we compared the osmolarity and density of different solutes required to lower the polar ratio to 1.0 (Table 1). In general, the low molecular mass compounds reduce the polar ratio to 1.0 at high osmolarities and negligible densities, whereas the high molecular mass solutes reduce the polar ratio to 1.0 at high densities and insignificant osmolarities.

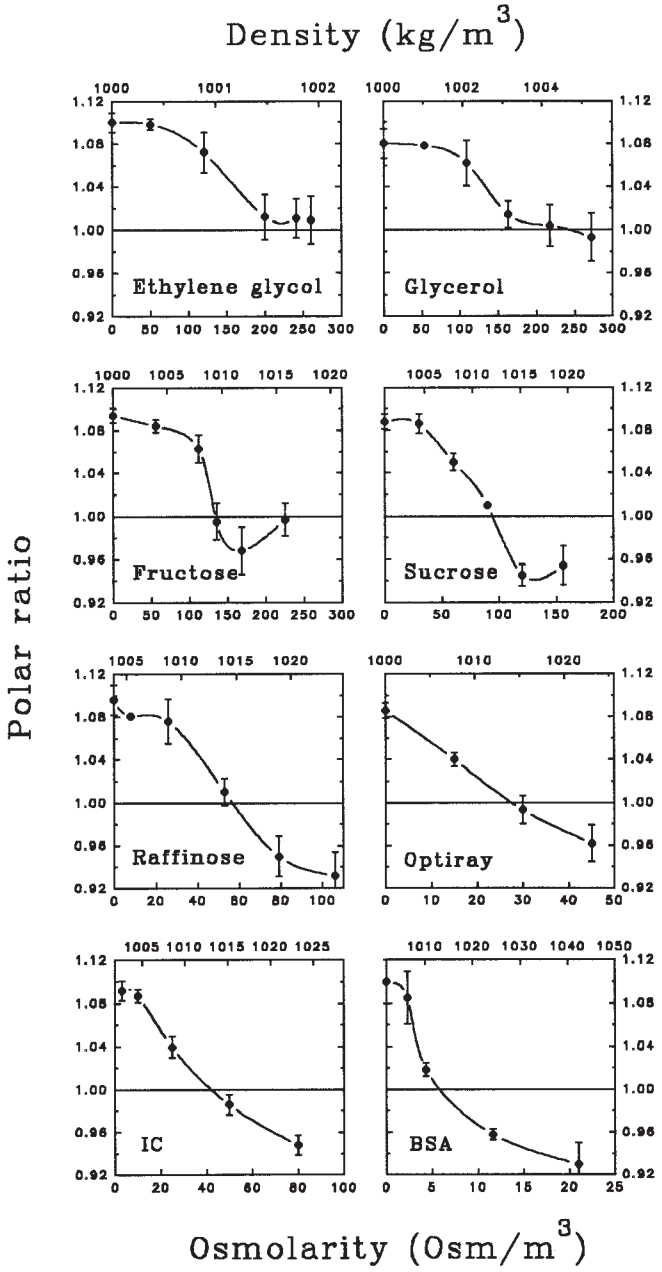


Fig. 3. The polar ratio of *Chara* internodal cells as a function of the osmolarity and density of the external medium. Notice that with increasing molecular mass of the solute, the range of the density abscissae expand, whereas those for osmolarity contract. Mean values ± SE are presented (N = 4–8).

In order to relate the osmolarities and densities of the external medium to those of the cell, we measured the osmolarity and density of the protoplasts of *Chara* internodal cells. The protoplasts of *Chara* internodal cells have an osmolarity of 233 ± 11 Osm/m^3 (0.577 MPa, N = 6) and density of 1015 ± 4 kg/m^3 (N = 20).

TABLE 1. The molecular mass of each solute and the osmolarity and density of the solutions required to decrease the polar ratio to 1.0.

Solute	Molecular mass (Da)	Osmolarity[a] (Osm/m³ [mOsm])	Density[b] (kg/m³ [g/mL × 10^{-3}])
Ethylene glycol	62.07	223	1002.3
Glycerol	92.09	235	1004.9
Fructose	180.16	132	1009.1
Sucrose	342.3	96	1012
Raffinose	594.5	56.6	1014.5
Optiray	807.13	27.5	1014.1
$Ca(BTIB)_2$	1226	42.3	1013.9
BSA	66 000	6.0	1014.2

[a] As a reference, the cellular osmolarity is 233 ± 11 Osm/m³ (N = 6).

[b] As a reference, the density of the protoplast is 1015 ± 4 kg/m³ (N = 20). Osmolarity, or osmotic concentration (c_s, in Osmol/m³), may be converted to osmotic pressure (π_s, in pascals) by using the following relation: $\pi_s = RTc_s$, where R is the gas constant (8.31 Pa·m³·mol^{-1}·K^{-1}) and T is the temperature in Kelvin.

Some planktonic blue-green algae regulate their density with gas vacuoles to control their position in the water column (Bold and Wynne, 1978), but there is no known case of density homeostasis in other cells, and an ad hoc invocation of such a phenomenon seems unwarranted at present. However, we tested whether *Chara* internodal cells vary their density in response to the density of the external medium. To this end, we measured the density of the protoplasts of internodal cells after a 2-h incubation in either 50 Osm/m³ (0.124 MPa) glycerol (density = 1001 kg/m³) or 50 Osm/m³ (0.124 MPa) $Ca(BTIB)_2$ (density = 1015 kg/m³). While the densities of the protoplasts incubated in the 50 Osm/m³ (0.124 MPa) solutions are slightly higher than those in APW alone (0.6 Osm/m³, 1.49 kPa), there is no significant difference in the densities of protoplasts incubated in glycerol or $Ca(BTIB)_2$ (Fig. 4), indicating that density homeostasis does not occur in *Chara*.

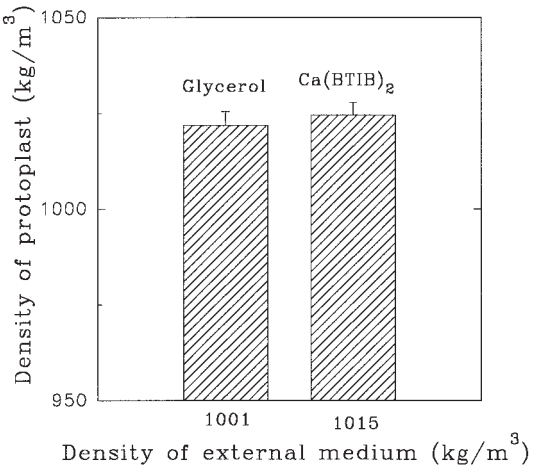


Fig. 4. The densities of protoplasts of *Chara* internodal cells incubated (for 2 h) in either 50 Osm/m³ (0.124 MPa) glycerol (density = 1001 kg/m³) or 50 Osm/m³ (0.124 MPa) $Ca(BTIB)_2$ (density = 1015 kg/m³). There is no significant difference in protoplast density between cells incubated in glycerol (1022 kg/m³; N = 18) or $Ca(BTIB)_2$ (1025 kg/m³; N = 18), P = 0.61. Note that the density of the protoplast increases from 1015 kg/m³ to 1022–1025 kg/m³ as the osmolarity of the medium increases, a phenomenon indicative of osmotic regulation (Tazawa, 1972; Bisson et al., 1995), but not density regulation.

DISCUSSION

Each of the solutes tested affects the ability of *Chara* internodal cells to sense gravity. This is what is predicted by the gravitational pressure theory if and only if the density is a factor. Each solute reduces the polar ratio to 1.0 at some concentration, but how can we determine whether the density or osmolarity of the external medium is responsible for the change in the gravity response? We will conduct two brief *Gedankenexperimenten.* First, let us choose a chemical that will exert its effect mainly by changing the density of the medium. Figure 2 indicates that, of all the solutes tested, bovine serum albumin (BSA) has the highest density and lowest osmolarity at any given concentration. Thus, of all the solutes tested, BSA is most likely to affect gravity sensing on the basis of its density. If we examine the data from the BSA experiments (Fig. 3, Table 1) we see that when cells are placed in a medium with a density of 1014.2 kg/m³ (osmolarity = 6 mol/m³ ≈ 14.9 kPa) the polar ratio is lowered to 1.0. If density were the only factor influencing gravisensing, then each of the other solutes would lower the polar ratio to 1.0 at a density of 1014.2 kg/m³. Thus, a plot of the density of each solute required to lower the polar ratio to 1.0, as a function of the molecular mass of the solute, would describe a horizontal line with a y-intercept at 1014.2 kg/m³ (filled circles, Fig. 5A). On the other hand, the corresponding osmolarities required to decrease the polar ratio to 1.0 would decrease with molecular mass (open circles, Fig. 5A).

Next, let us select a chemical that will have a predominantly osmotic effect on the external medium. Figure 1 indicates that ethylene glycol is the solute most likely to exert a purely osmotic effect on gravisensing. The data from the ethylene glycol experiments (Fig. 3, Table 1) indicate that a 223 Osm/m³ (0.55 MPa) solution (density = 1002.3 kg/m³) reduces the polar ratio to 1.0. Thus, if osmolarity were the only component to affect gravity sensing, a plot of the osmolarity of each solute required to lower the polar ratio to 1.0 would show a horizontal line with a y-intercept at 223 Osm/m³ (0.55 MPa, open circles, Fig. 5B), while the corresponding points representing the densities of the solutions required to lower the polar ratio to 1.0 would increase with molecular mass (closed circles, Fig. 5B).

Figure 6 shows the observed experimental results. It is

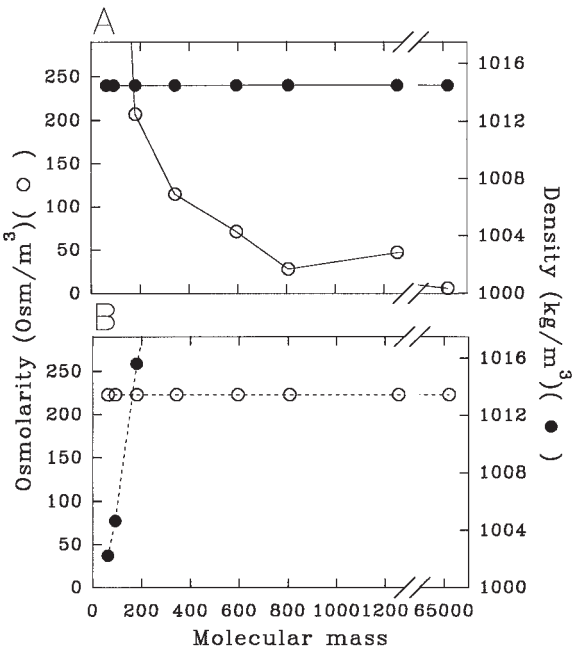


Fig. 5. The results of two *Gedankenexperimenten* in which the concentration of each solute (expressed as the osmolarity and density of the solution) required to reduce the polar ratio to 1.0 plotted against molecular mass assuming (A) that density is the only property of the medium influencing gravity sensing, and (B) that osmolarity is the only property of the medium affecting gravisensing. Note that in (A) the points representing the osmolarities of the ethylene glycol and glycerol solutions required to achieve a density of 1014.2 kg/m^3 are out of range. Similarly, only the points representing the densities of 223 Osm/m^3 (0.552 MPa) solutions of ethylene glycol, glycerol, and fructose fall within the range of (B). The densities were calculated from the osmolarities and the regressions in Fig. 2.

evident that neither result of the *Gedankenexperimenten* alone describes the effects of all the solutes on the gravity-induced polarity of cytoplasmic streaming in *Chara*. In fact, Fig. 6 is a hybrid of Fig. 5a and b. The points corresponding to the four solutes of highest molecular mass in Fig. 6 are almost identical to those in Fig. 5a, whereas the points for ethylene glycol and glycerol, the solutes shown in Fig. 6 with the lowest molecular mass, are the same as those in Fig. 5b. The densities and osmolarities of fructose and sucrose required to lower the polar ratio to 1.0 (Fig. 6) fall between the corresponding values from the two *Gedankenexperimenten* illustrated in Fig. 5a and b. These results indicate that *both* osmolarity and density affect gravity sensing. We suggest that compounds to the left of the intersection of the density and osmolarity lines (Fig. 6) affect gravity sensing mainly through an osmotic effect, and those compounds to the right of the intersection have predominantly a density effect. Since fructose lies near the crossover point on Fig. 6, the osmolarity and density of fructose solutions (and those of impermeant molecules with a similar molecular mass) may affect gravity perception almost equally.

We interpret these data to mean that high molecular mass solutes probably affect the gravity response by changing the static buoyancy of the protoplast. This interpretation is supported by the fact that the density of the external medium required to abolish the gravity response plateaus at ~1014 kg/m^3 (Fig. 6), which is about the density of the protoplast measured gravimetrically (1015 ± 4 kg/m^3). At this density, the osmolarities of raffinose, Optiray, Ca(BTIB)$_2$, and BSA vary over an order of magnitude and are too low to have an effect on the gravity response in the absence of a density component (see osmolarity data for ethylene glycol and glycerol, Fig. 3). The effect of these high molecular mass molecules on the polar ratio is consistent with the gravitational pressure theory of gravisensing.

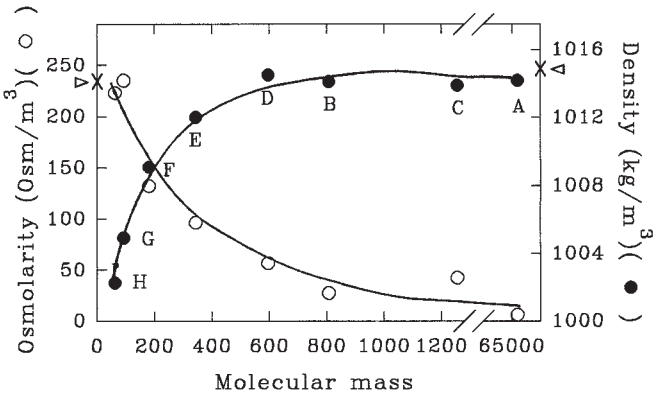


Fig. 6. The concentration of each solute (expressed as the osmolarity and density of the solution) required to reduce the polar ratio to 1.0 (see Table 1) plotted against the molecular mass of the solute.

While the density effect is important for high molecular mass solutes, we suspect that low molecular mass solutes affect gravity sensing by increasing the osmolarity of the external medium. This will decrease the turgor of the cell. The reduction of turgor may disrupt the attachment or function of integrin-like proteins, which act as gravireceptors and span the plasma membrane-ECM junction (Wayne, Staves, and Leopold, 1992). Alternatively, reducing the turgor of the cell will decrease the strain in the plasma membrane. A strained plasma membrane could be required to enable the cell to respond to small differences in pressure caused by gravity (Staves et al., 1992).

Interestingly, the osmolarities of the ethylene glycol and glycerol solutions required to lower the polar ratio to 1.0 are close to the osmolarity of *Chara* internodal cells (233 ± 11 Osm/m^3 ≈ 0.577 MPa). In order to evaluate the effect of osmolarity on turgor, we must calculate the osmotic pressure due to the solute. The osmotic pressure (π_s) of a solution may be calculated from its osmotic concentration (c_s, in Osmol/m^3) by using the van't Hoff equation as generalized by Planck (1927):

$$\pi_s = RTc_s$$

where R is the gas constant (8.31 Pa · m^3 · mol^{-1} · K^{-1}) and T is the temperature in Kelvin.

Thus the osmotic pressure of the external medium is directly proportional to the concentration of the solutes. An increase in the solute concentration in the external medium decreases the turgor pressure (P) of the cell in a nonlinear manner described by the following relation:

$$P = \sigma_s(\pi_i - RTc_s)$$

where π_i is the osmotic pressure inside the cell and σ_s is

the reflection coefficient of the membrane to the solute in the medium. Since the reflection coefficient of all solutes tested is 1 (Overton, 1899; Collander, 1949, 1954, 1959; Steudle and Zimmermann, 1974; Steudle and Tyerman, 1983; Staves, Wayne, and Leopold, 1992; Wayne, Mimura, and Shimmen, 1994), and the osmolarity of the cells is essentially constant at 233 Osm/m^3, turgor is only affected by the osmotic concentration in the external medium. We see that gravisensing is dependent on turgor and is abolished by external low molecular mass solutes when the turgor pressure approaches zero.

It seems quite clear that a variety of molecules, acting externally on the cell, affect the gravity-induced polarity of cytoplasmic streaming in *Chara* internodal cells. Because the molecules tested have different chemical properties, we assume that their effects on the polar ratio result only from their physical (e.g., colligative and hydrostatic) properties.

Francis Darwin, in his opening address to the British Association at Cambridge (1904), wrote ''it must be clearly pointed out that for a large number of plants, such as Algae and Fungi, no statoliths are known to exist, though their complete absence has not been proved. Here we must either believe in Noll's minute and hitherto unseen statoliths or in a different mechanism, such as hydrostatic pressure.'' The experiments described in this paper demonstrate that ''minute and hitherto unseen statoliths'' cannot plausibly account for graviresponsiveness in *Chara* since changes in the density of the external medium will not affect the buoyancy of intracellular particles, seen or unseen. These results support Darwin's alternative to the statolith theory, i.e., that the density of the external medium affects gravity sensing by changing the buoyancy of the protoplast within the ECM. We propose that under normal conditions the protoplast settles within the ECM, resulting in a differential tension and compression between the plasma membrane and the ECM at the top and bottom of the cell, respectively. These differential pressures activate the gravireceptors, at the top and bottom of the cell, which we suggest are integrin-like proteins that span the plasma membrane–ECM junction. If the density of the external medium is greater than that of the protoplast, the protoplast becomes buoyant, resulting in a compression between the plasma membrane and ECM at the top of the cell and a tension between the plasma membrane and the bottom of the cell. This inversion of the normal situation results in a reversal of the gravity-induced polarity of cytoplasmic streaming. In summary, we conclude that in *Chara* internodal cells, the whole cell (not intracellular particles) must function as the gravity sensor.

Might the density of external solutes also affect the gravity response of a plant organ which contains statoliths? We are presently examining the inhibition of gravitropic curvature of the primary roots of rice seedlings caused by increasing the density of the external medium. These results will be reported elsewhere in this journal.

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