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# Membrane voltage of marine phytoplankton, measured in the diatom *Coscinodiscus radiatus*

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**Abstract** In order to investigate the role of the ionic relations in buoyancy of marine phytoplankton, voltage recordings have been made on the planktonic diatom *Coscinodiscus radiatus* using conventional glass microelectrode techniques. The most negative Nernst equilibrium voltage in *C. radiatus* is $E_K$, the potassium equilibrium voltage of around −85 mV. Accordingly, stable voltages of −40 to −80 mV were recorded from *C. radiatus* which conforms to the general theory of electro-diffusion of ions through membranes (voltage range $V_d$). In addition, membrane voltages much more negative, e.g. up to −140 mV (voltage range $V_p$), have been recorded in *C. radiatus*; these voltages demonstrate the operation of an electrogenic pump. Within the voltage range $V_d$, light-on and -off (microscope illumination) caused weak hyper- and depolarizations by about 2 mV with a time constant of about 10 s. Also within $V_d$, spontaneous oscillations could be observed with a frequency of about 0.03 Hz and irregular amplitudes up to 30 mV. These phenomena are simulated by a model for electrocoupling of the major ion transporters in plants, as worked out for guard cells with their subtle osmoregulatory system. Equivalent mechanisms are suggested to operate in planktonic diatoms for adjustment of buoyancy by appropriate uptake and release of ions.

## Introduction

The processes of sinking and sedimentation of marine phytoplankton transfer large quantities of photosynthetic carbon from the atmosphere and surface waters to deep waters and ultimately to the lithosphere. In the case of non-motile cells, such as diatoms, buoyancy is one of the factors which determine the actual amount of this transfer. Gross and Zeuthen (1948) have suggested that these cells control their buoyancy by appropriate exchange of heavy and light ions with the external sea water.

Since the voltage $V_m$ across the plasma membrane is essential as part of the driving force for sign and amount of any charge movement across the plasma membrane, $V_m$ is expected to play a predominant role in the context of this hypothesis.

In the period of the 1960's there was a major effort in several laboratories to investigate the ionic relations of marine algae. However, this effort ceased, mainly because $V_m$ of all the investigated cells behaved rather boringly, remaining near $E_K$, the equilibrium voltage for $K^+$ diffusion, without noticeable variation upon changes in physiological conditions (Gutknecht and Dainty 1968). In contrast, consecutive investigations of the giant marine algal cells of *Acetabularia acetabulum* (Gradmann 1970; Saddler 1970) did show a challenging variety of electrophysiological effects, in particular, changes in $V_m$.

In all these investigations, impaled microelectrodes were used; these electrodes are known to introduce a considerable leak conductance at the interface between the glass pipette and the penetrated membrane. Since such a local leak does not matter as much in large cells compared to small cells, the findings in *Acetabularia acetabulum* can be considered more reliable. This view is supported by improvements of microelectro-techniques for application in small cells and by the introduction of patch-clamp techniques with seal resistances of many GΩ (Hamill et al. 1981) between pipette and membrane. Based on improved measurements, the recent understanding of physiological interaction between $V_m$ and ionic relations in plants may be summarized as follows (for details see Gradmann et al. 1993).

The voltage across the plasma membrane of plants is, in general, bistable. There is one stable voltage range, $V_d$,

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slightly more positive than $E_K$, the equilibrium voltage of potassium; in this range the electrical properties seem to obey the classic theory of electro-diffusion of ions through membranes. Accordingly, when a cell shows a resting voltage more positive than $E_K$, usually the most negative Nernst diffusion voltage for a plant cell, the cell is said to be in a "diffusion state" or in the K-state.

The other range of stable membrane voltages, $V_p$, is usually much more negative than $E_K$; this range is outside the validity of the concept of electro-diffusion and is accepted to be maintained by the additional operation of an active ion transporter, usually an $H^+$-extruding p-type ATPase, briefly called the $H^+$ pump. Correspondingly, a cell is said to be in the "pump state" when its resting voltage is much more negative than $E_K$. Between $V_d$ and $V_p$, there is a range $V_o$ including $E_K$, which is attained rather rarely; membrane voltages in $V_o$ tend to change either to $V_d$ or to $V_p$.

In this context it has been pointed out (Gradmann et al. 1993) that plant cells take up ions when they are in the pump state and lose ions when they are in the diffusion state; as far as the most permeable $K^+$ is concerned, this notion is almost trivial. As a corollary, there is no steady state in which the ion balance is equilibrated; plant cells rather switch in appropriate intervals between the diffusion state and the pump state to achieve a long term balance of ionic relations. This concept has been worked out in some detail for the guard cells that control the opening and closing of leaf stomata; these cells must operate a rather sophisticated regulatory system for tuning their osmotic state, hence their turgor and shape.

Since such a concept of ion regulation should also be applicable to marine algae, especially planktonic diatoms with their problem of buoyancy regulation, experimental evidence is required before mechanistic details of the elegant Gross and Zeuthen (1948) hypothesis can be examined. It was the aim of the present study to provide this evidence.

In the early days of plant electrophysiology, the membrane voltage of intact cells was focussed upon. When patch-clamp techniques were introduced, our interest shifted from voltage in intact cells to currents through cell-wall-free membranes. After some vain attempts to do patch-clamp experiments on protoplasts of marine diatoms, and after the above rediscovery of the impact of membrane voltage on ionic relations of intact plant cells, we focussed again on the membrane voltage of intact cells. This is the first documentation of membrane voltages in diatoms.

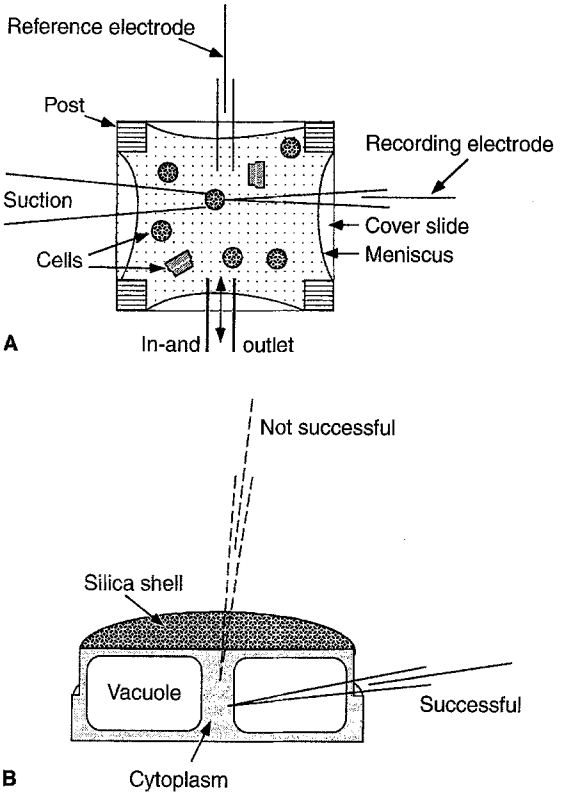


**Fig. 1** Experimental setup: **A** schematic top view of experimental chamber with cells. **B** schematic anatomy of a cell with position of voltage recording electrode

## Materials and methods

### Cells

Axenic cultures of the following marine diatoms were obtained from the Provasoli-Guillard Center for Culture of Marine Phytoplankton. All cultures were maintained in *f*/2 medium (Guillard and Ryther 1962). Cultures examined were: *Achnanthes* sp. (CCMP 101); *Bellerochea* sp. (CCMP 144); *Coscinodiscus radiatus* (CCMP 310); *Cylindrotheca closterium* (CCMP 341); *Ditylum brightwellii* (CCMP 358); *Leptocylindrus danicus* (CCMP 469); *Melosira nummuloides* (CCMP 482); *Odontella mobiliensis* (CCMP 596); *Porosira glacialis* (CCMP 651); *Stephanopyxis palmeriana* (CCMP 814); *Thalassiosira weissflogii* (CCMP 1336). These species were examined microscopically for their potential to serve for electrophysiological investigations. The most promising candidates were *O. mobiliensis*, *S. palmeriana*, and *C. radiatus*. After some preliminary recordings, the somewhat smaller cells of *O. mobiliensis* (ca. $50 \times 20$ µm) and of *S. palmeriana* (ca. $30 \times 40$ µm) were discarded because no stable voltage recordings could be achieved with them. Therefore, all recordings reported here were gained from *C. radiatus* (ca. $70 \times 30$ µm). The experimental approach is shown schematically by Fig. 1.

The silicate frustule of *Coscinodiscus radiatus* has the classic Petri dish shape of a centric diatom and encloses a protoplast that is comprised of cytoplasm (about 20% of the protoplast volume) and a main vacuole (about 80%). This vacuole forms a torus surrounding a central cytoplasmic columella which extends from hypotheca to epitheca.

### Setup

Standard glass microelectrode techniques have been applied with Leitz micromanipulators and a Nikon TMD Diaphot inverted microscope on a vibration isolated table. The measuring chamber was formed from the bottom of a 5 cm polystyrene Petri dish with four ($2 \times 2 \times 3$ mm) acrylic posts which supported a $10 \times 10$ mm coverslide at the corners. From one side of the chamber a blunt fused

pipette (about 20 µm width) was introduced as a holding pipette or barrier at such an angle that the tip could touch the floor of the chamber. The measuring pipette was introduced about symmetrically from the other side. The reference electrode (silver-silver chloride) was positioned between the two rear posts, and between the two front posts the medium and cells could be introduced with a pasteur pipette. Due to the plane-optical properties of the measuring chamber, the cells on the floor could be well observed. External medium was usually the culture medium (Guillard's *f*/2) or filtered sea water, sometimes diluted by about 10% in order to separate the two thecae of the frustule osmotically for improved access of the measuring pipette to the protoplast.

Glass pipettes were pulled from filamented boro-silicate glass (Dagan FMG 15) on a List vertical pipette puller in two steps to achieve sharp, non-bending tips for penetration of the silica frustule. In most experiments the pipettes were filled with 400 m*M* KCl and 1 m*M* EGTA (ethyleneglycol-bis-tetraacetic acid) to approximate the ionic conditions of the cytoplasm in marine algae (about 400 m*M* KCl and $<1$ µ*M* free $Ca^{2+}$) (Gutknecht and Dainty 1968). The resistance of the voltage recording pipettes in sea water was about 10 MΩ. The voltage was monitored by a Dagan 3900 integrating patch-clamp amplifier in the current-clamp mode ($I_0$), displayed on an oscilloscope (for identification of excess noise) and recorded on a strip chart recorder.

## Results

### Microscopic observations

Gentle insertion of the microelectrodes through the silicate shell into the cytoplasm was tried in various geometric configurations. In particular, we tried to approach the central mass of cytoplasm in the columella by penetration of the center of the shell. However, stable voltage recordings over several minutes were only obtained by lateral insertion of the electrode (see Fig. 1B).

According to Fig. 1B, the electrode tips must have frequently been located in the vacuolar compartment. However, when the electrode appeared to be in the vacuole, no voltage different from zero was recorded. Consequently, we examined the physiological state of the cells by staining with $10^{-7}$ *M* neutral red. We found the expected dark staining of the acid vacuoles in only about 5% of the population. In the majority of cells, the volume of the vacuole was as transparent as the external sea water (pH 8), indicating that a minor fraction of healthy cells was sufficient to maintain a growing culture. Interestingly, when the main vacuole was apparently filled with sea water, many small vacuoles in the cytoplasm could be identified by the dark red stain characteristic of neutral red in acidic environment.

It was frequently observed that the cytoplasm contracted to a small droplet within a few seconds after the electrode penetrated the shell. Our interpretation of this event is that $Ca^{2+}$ leaked into the cell during penetration and elevated the concentration of free $Ca^{2+}$ in the cytoplasm far above the physiological range, causing a global over-reaction of the contractile apparatus. No stable voltage recordings were obtained from such cytoplasmic droplets.

Most stable recordings were obtained with the configuration sketched in Fig. 1B, where the electrode tip was advanced through the vacuole into the central cytoplasm, after penetration of the silica shell. This can be seen in the recordings of Figs. 2, 3A and B where the main component of the signal (from the central cytoplasm) is preceded by a short negative transient (from the peripheric cytoplasm) followed by a short period of zero voltage (recorded in the vacuolar space). In rare cases, stable voltages could be recorded immediately from the thin cytoplasmic layer under the silicate frustule.

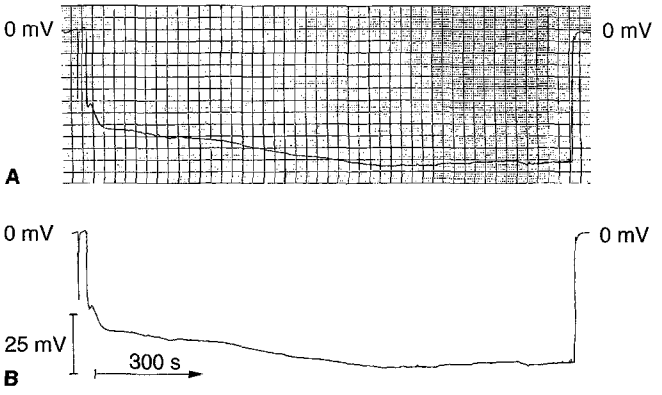


**Fig. 2** *Coscinodiscus radiatus.* Typical original recording **A** and reproduction **B** of membrane voltage. Note: transient polarization (in peripheric cytoplasm) and short period of zero voltage (in vacuole) before main recording, characteristic swerve at beginning of main recording, steady voltage level around $-60$ mV, and abrupt end of recording

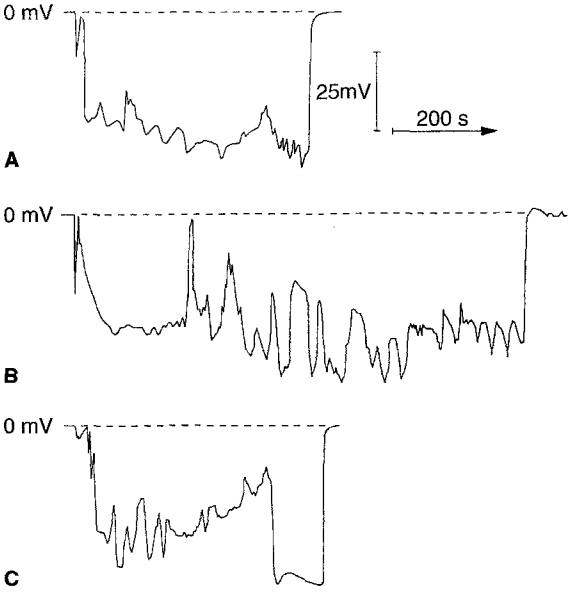


**Fig. 3** *Coscinodiscus radiatus.* Three examples, **A**, **B** and **C**, of spontaneous oscillations of membrane voltage. Note: frequency about 0.03 Hz; shapes and amplitudes irregular ranging from a few mV up to about 30 mV; in tracing C, abrupt transition from oscillations to steady-state

## Voltage recordings

### *Qualitative features*

Fig. 2 shows an example of an original voltage recording from *Coscinodicus radiatus* and of a corresponding reproduction as used here for presentation purposes. Upon successful impalement of the electrode in the cytoplasm, the recorded voltage jumped from zero to about $-40$ mV and approached the more negative level in the voltage range $V_d$ by a sigmoid time course within about 2 min. Within about the first 30 s after the initial jump at successful impalement, there was a transient depolarization which can be found in many original voltage recordings from cells of plants (e.g. Blatt 1987) and fungi (e.g. Slayman 1965). In our case, these transients appeared in various degrees, ranging from a faint shoulder as in Fig. 4, to clear transient depolarization as in Fig. 2. The end of a recording was usually an abrupt step to zero voltage (see Figs. 2 and 3), probably when the cell eventually succeeded in forming an exocytotic membrane against the invading electrode tip. Reimpalement was usually not successful.

### *Steady state*

Stable voltages over several minutes have been recorded from about $-40$ to $-80$ mV, i.e. within the voltage range $V_d$. Standard statistics yield a mean of about $-55$ mV $\pm 10$ mV SD. Since, however, low voltages are considered to result from a leakage artifact around the inserted tip, the more negative voltages are assumed to be more representative of an intact cell.

## Effect of light

The effect of light on the voltage was small. Fig. 4 shows some examples. Turning on the illumination of the microscope (about 1 W m$^{-2}$ white light) caused a roughly exponential hyperpolarization by a $\Delta V_\infty$ of about $-2$ mV with a time constant $\tau$ of about 10 s: $\Delta V(t) = \Delta V_\infty [1 - \exp(-t/\tau)]$; turning off the light caused a corresponding depolarization to the dark level.

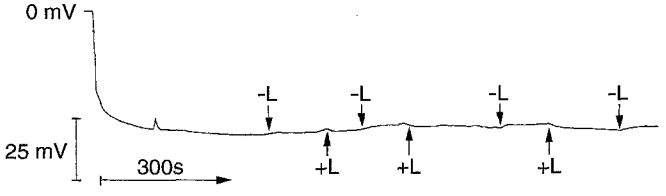


**Fig. 4** *Coscinodiscus radiatus.* Effect of light on membrane voltage. Small polarization by about $-2$ mV upon light (microscope illumination) on ($+L$) and symmetric depolarization to dark level upon light off ($-L$)

### *Oscillations*

Apart from the steady state recordings, fluctuations were rather common. In quite a few of these fluctuations, oscillations could be identified. Some examples are given in Fig. 3. The duration of a period was about 30 s regularly. The amplitudes, however, were rather irregular, ranging from a few mV up to about 30 mV. No reasons have been found which caused the membrane voltage to change between a stable state and an oscillating one. Fig. 3C shows a spontaneous transition from periodic fluctuations to a stable recording.

### *Voltages exceeding diffusion voltage*

Gutknecht and Dainty (1968) reported unpublished results from H. Kessler, indicating the $K^+$ concentration in *Coscinodiscus wailesii* to be 461 m*M*. With an activity coefficient of 0.65 at this concentration (CRC Handbook of Chemistry and Physics, 52 edn), the internal activity is about 300 m*M*, and the Nernst equilibrium voltage for $K^+$ diffusion, $E_K$, is $-85$ mV, relative to a normal $K^+$ concentration of 10 m*M* in the external sea water. This voltage is the most negative diffusion voltage of the major ions and marks, therefore, the theoretical limit of the range $V_d$ of membrane voltages which can be explained by the classic theory of electrodiffusion of ions through membranes. Voltages which

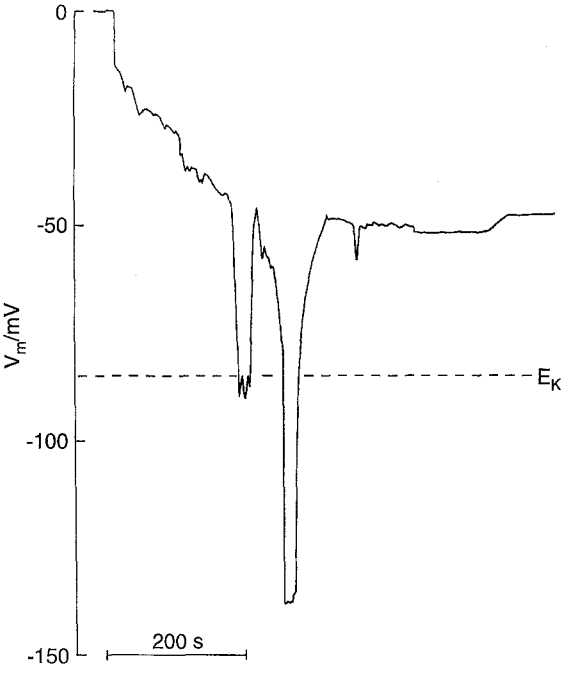


**Fig. 5** *Coscinodiscus radiatus.* Two episodes of markedly negative voltage, $< E_K$, from one recording. $E_K = -85$ mV marks negative limit of $V_d$ consistent with the theory of electrodiffusion of ions; conclusion: an electrogenic pump is causing $V_m$ to be more negative than $V_d$

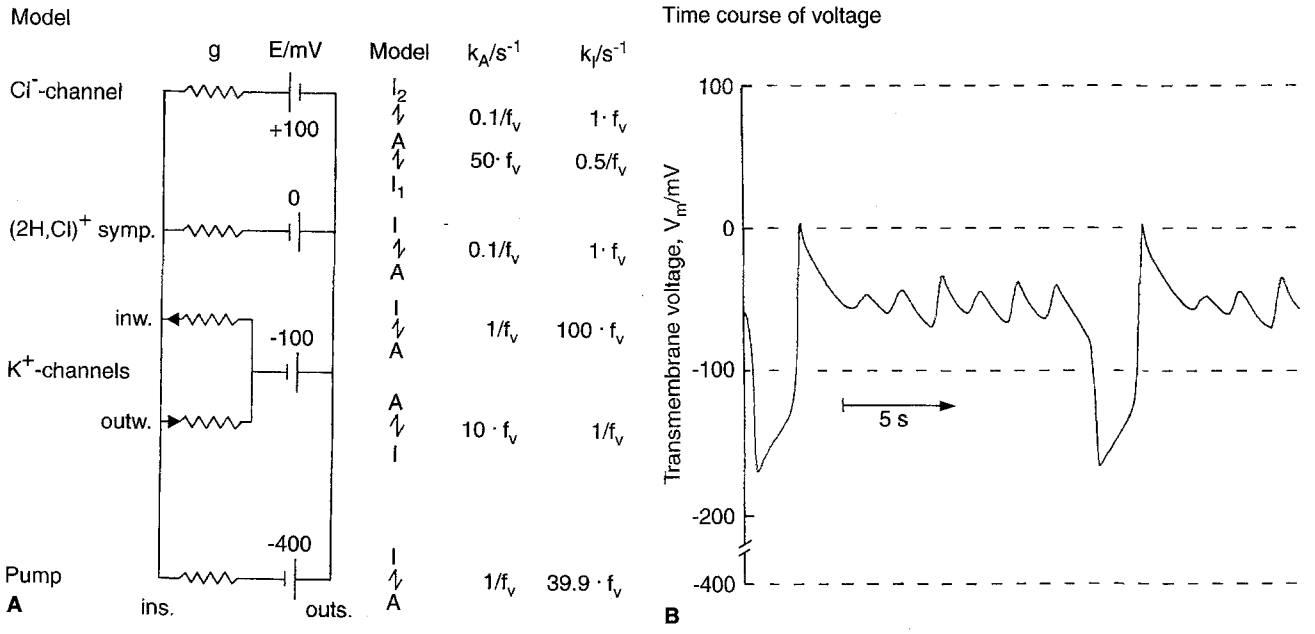


**Fig. 6** *Coscinodiscus radiatus.* Model simulation of measured voltage courses. **A** simplified synopsis of electrical behaviour (current/voltage/time-relationships) of major ion transporters in plant cells, according to Gradmann et al. (1993), explanations in "Discussion"; **B** predicted time course of free running voltage of the model with numerical parameters as listed; points of interest: occasional polarizations beyond $E_K$ and oscillations within $V_d$ with seemingly irregular amplitudes

are significantly more negative than $E_K$, namely in the range $V_p$, have been recorded in many cells of plants and fungi, where they indicate the operation of an electrogenic pump. This pump is usually a $H^+$-extruding p-type ATPase in which ion translocation is coupled directly with a metabolic reaction. We have recorded a few instances of similar negative membrane voltages in the range $V_p$ in *C. radiatus*. Fig. 5 shows two examples from one cell; about 3 min after impalement, the voltage showed a transient polarization to about −90 mV and shortly thereafter, a polarization to −140 mV which lasted for a few seconds before it moved again to the stable level around −60 mV in the range $V_d$.

## Discussion

In the "Introduction", we have pointed out that voltage recordings from small cells with conventional glass-microelectrodes are likely to be biased by the leak conductance between the electrode and the penetrated membrane. We have to assume that the absolute voltages given in the "Results" suffer from the same problem.

Nevertheless, all the qualitative results of the present study compare well with the general view of the electrical properties of plant membranes, recently expressed in terms of a model (Gradmann et al. 1993). In order to discuss this matter, the model (Fig. 6A), based on experimental data from plants and fungi, is briefly described.

A simple physical analog for the electrical properties of a membrane is a parallel arrangement of ion transporters which act as batteries, with their electromotive forces $E_i$ and their conductances $g_i$. The major ion transporters are those that can exert a significant effect on the transmembrane voltage $V_m = \Sigma E_i g_i / \Sigma g_i$. $Ca^{2+}$ channels, for instance, do not belong to this group because they pass only catalytic amounts of substrate. Five well known ion transporters are considered to be *major*: an $H^+$ pump, two types of $K^+$ channels (one outward rectifying and one inward rectifying), a $Cl^-$ channel which catalyses normally only $Cl^-$ release according to the outward-directed electrochemical gradient for $Cl^-$ under physiological conditions, and a $(2H^+, Cl^-)^+$ symporter which enables the membrane to import $Cl^-$ uphill by the driving force of the electrochemical $H^+$ gradient. The conductances of all these transporters depend on $V_m$ in a way that can be approximated by the following formalism: each transporter can switch via a symmetric Eyring barrier between an active State A (maximum conductance) and an inactive State I, with the transition probabilities (in $s^{-1}$) $k_A$ for activation and $k_I$ for inactivation. Two inactive states ($I_1$ and $I_2$) must be considered for the $Cl^-$ channel. With some simplifying assumptions, the voltage dependences of each transporter enter the system by the respective transition probabilities $k_A = k_A^0/f_v$ and $k_I = k_I^0/f_v$ (if positive voltage would drive the system into the active state, or vice versa $k_A = k_A^0 f_v$ and $k_I = k_I^0 f_v$), where $f_v = \exp[V_m F/(2RT)]$ is a factor describing the voltage-dependence ($R$, $T$ and $F$ having their

usual thermodynamic meanings) and the superscript $^0$ denotes the values of $k_A$ or $k_I$ at zero voltage. In this model, voltage changes cause conductance changes with the velocities given by the $k$'s, and the conductance changes, in turn, will cause voltage changes, and so on. Depending on the actual values of $k^0$, the system can approach a steady state or can oscillate.

The model with the numerical parameters given in Fig. 6A yields the time course of the free running voltage as depicted in Fig. 6B. This simulated voltage change shows several features which have been found in our voltage recordings from *Coscinodiscus radiatus*, namely relatively short excursions from $V_d$ to $V_p$ (see Fig. 5) and oscillations in the $V_d$ range with seemingly irregular amplitudes, but a well defined frequency (see Fig. 3). The difference in the time scale between the experimental data and the model data could easily be accounted for by multiplying all $k$ values by a common factor (about 0.05); this has not been done in order to keep the model data simple.

We do not claim that the ionic relations of *Coscinodiscus radiatus* are reflected numerically in the model; our comparison with the model serves only to point out that the observed phenomena are consistent with the basic findings from plants and fungi. In particular, our results from *C. radiatus* show striking similarities to the results from guard cells (for which the model was originally designed), with their efficient oscillating mechanisms for osmotic tuning achieved by appropriate switching between uptake of ions in $V_p$ and release in $V_d$.

At the present time, the data from *Coscinodiscus radiatus* are not sufficient to verify an equivalent mechanism for the control of buoyancy. On the other hand, our results render the hypothesis likely that this organism employs the same ionic machinery for buoyancy control as guard cells do for the control of their volume by turgor.

## Conclusions

The presented voltage recordings of *Coscinodiscus radiatus* support the idea that marine diatoms can adjust their buoyancy by the same machinery of electrocoupled ion transporters, which enables plant cells, in particular guard cells, to control their turgor.

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