Chapter 6

Cell Biophysics and Plant Gravitropism

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ABSTRACT

Biological systems respond to environmental stimuli with the following sequence of events: (1) the signal is received; (2) membrane depolarization occurs; (3) messenger molecules are released and the biological response occurs. Zea mays (corn) seedlings respond to the gravity stimulus with the same sequence elaborated into a working hypothesis: (1) the gravitational vector is perceived by a statolith or by an unknown sensor system; (2) ion and hormone gating channels connecting vascular to surrounding tissues are depolarized; (3) the asymmetrically released ions and hormones initiate a cascade of reactions resulting in asymmetric growth. Mechanisms for amplification of the weak gravity signal are discussed. An experiment is proposed for distinguishing between statolith detection of the gravity vector and possible alternative detectors.

INTRODUCTION

If turmoil is a sign of progress and the number of meetings held recently reflects turmoil, then we must be making a great deal of progress toward understanding gravitational effects on the cell. This is a unique opportunity to ask important questions relating to the coupling of gravity to biological phenomena. We are trying to couple very weak gravitational forces to cellular functions which require strong forces, and this is a difficult task. This chapter will emphasize the following single theme — in every case where a signal is received by a cell or biological system, the first effect is a change in the membrane potential. In every case that has been carefully examined, (1) the signal is received; (2) one or more membranes are depolarized; and (3) there is a subsequent response.

If this sequence is universal the real questions become: (1) how is gravity coupled to changes in membrane potentials; to wit, how does the gravity stimulus cause membrane depolarization; and, (2) how does the organism use changed membrane potentials, after amplification, to give meaningful biological responses? By formulating these questions, I have given my own work and a lot of other work an archaeological perspective, since most of us are working on later biological responses rather than on the earlier gravity perception and transduction. The whole question of growth and hormone metabolism belongs to more recent events in gravitational archaeology. If we want to look at origins, we should be determining how gravity is coupled to the depolarization and how depolarization is used for very early events.

The following presentation will briefly cover the ubiquity of gravity and how it can lead to the concept of multiple detectors. It will then describe how biology can use kT (Brownian motion) rather than be defeated by kT — that is, biology has actually learned to use kT. The discussion will include multiple gravity detectors and what can be learned from light reception and perception; the transduction systems of Phycomyces and Chlamydomonas; my own work with corn (Zea mays) seedlings; chemotaxis; and, finally, something about coupling.

Here the very obvious and ubiquitous nature of gravity has led to a trivial concept concerning the response of a biological system to gravity. For example, in the comic strip “B.C.” the question is asked: “How come the branches of a tree grow up and the roots grow down?” Thor answers: “Cause roots need dirt and branches need sky.” Scientists may have overlooked biological responses to gravity because of their very commonplace nature and because we have not been able to employ gravity-free controls.

COUPLING OF WEAK FORCES

Enzymes have learned to break 90 kilocalorie (kcal/mole) bonds but accomplish this by using only one kilocalorie bonds. Enzymes employ hydrogen bonding, hydrophobic interactions, ionic interactions, and van der Waal’s forces. All of these forces, even the ionic forces in water, are of the order of one kilocalorie/mole. Enzymes use these little forces to break strong covalent bonds which are several orders of magnitude larger. What an enzyme is doing has been described by Careri (Careri, 1984; Alberts et al., 1983) in the following manner.

An example is found in the hydrolysis of an ester. The ester has two rigid domains, an alcohol and an acid domain. The enzyme also has two rigid domains. It adsorbs the ester, holds it in a constrained configuration, and waits until the enzyme-substrate complex is sufficiently bombarded by rapidly moving water molecules to break that bond. Thus,
nature uses heat energy to do chemical work even though the enzyme itself has available only very weak chemical forces. So, nature, rather than having to fight against kT, is using kT through enzymatic catalysis in a meaningful way. Low-energy inputs can achieve a big energy output by summing a large number of weak forces over a substantial period of time and accumulating the necessary thermal energy to do the work.

Another example comes from following electron flow in photosynthesis. Figure 1 illustrates the familiar Z scheme of photosynthesis. There is not enough energy in a quantum of red light to move an electron to a sufficiently negative potential to accomplish the reduction of a pyridine nucleotide. So, what does nature do? It gathers two photons and sums the energy content of the two photons. The first photon impacts a molecule of chlorophyll b and kicks an electron up into a conduction band and leaves behind a positive hole. That positive hole is a potent oxidant, capable of oxidizing water to oxygen. Meanwhile, the electron in the electron conduction band falls through a potential gradient, and as it does so, the energy liberated makes ATP. Another photon hits a chlorophyll a molecule, again kicking an electron up into a conduction band. The first electron that came from chlorophyll b now can drift down and fill that hole, that is, reduce the oxidant. The second electron is now at the potential of ferredoxin or a pyridine nucleotide, sufficient to accomplish this difficult reduction. In this example, nature is using two photons to do what one photon cannot do, and it is doing it by summing the two. These examples illustrate how nature can aggregate small forces to accomplish difficult tasks.

**MULTIPLICITY OF GRAVITY DETECTORS**

The lessons we have learned from experiments in light detection demonstrate multiple detectors for various colors and intensities of light and suggest that there may be multiple gravity detectors as well. Even without the comparative biochemical knowledge of light detection, we might anticipate multiple detectors of gravity — a pervasive force on all living creatures through all of time. Many light detectors have been identified in plants. For example, chlorophyll a and b are parts of the photosynthetic system with chlorophyll b playing the major light absorbing role in System II and chlorophyll a absorbing light for System I (Figure 1). Each is adapted to utilizing a certain portion of the spectrum. In addition, so-called “accessory” pigments, such as the carotenoids, are adapted to the capture of other wavelengths of light. They capture excitation energy at wavebands that chlorophyll cannot capture, and they transfer that excitation energy to chlorophyll.

Thus, light gives us an important precedent. Just as different pigments capture different wavelengths of light, gravity detectors may also include many devices for the detection of gravity and electromagnetic perturbations. This may be the kind of mechanism we should look for. In addition to using different wavelengths of light, nature has learned to use not only high-intensity light receptors, but also low-intensity receptors. Low-energy receptors, such as carotenoids and the flavines, are excited and subsequently control all of the tropisms. They receive minute amounts of blue light energy and transduce it into a huge growth response. Another kind of low-energy receptor is phyto-
BIOPHYSICS AND GRAVITROPISM

Figure 2. A multiple exposure, taken every 40 min, of the growth of the *Phycomyces* sporangiophore. (Photograph by D.S. Dennison. Reproduced, with permission, from Cerda-Olmedo, E. and Lipson, E.D., *Phycomyces*, Cold Spring Harbor Laboratory, 1987.)

chrome. Here a few red quanta can be amplified into a huge growth response by a very complex elaboration of the signal.

TROPIC SYSTEMS

Figure 2 illustrates the tropic system of the fungus *Phycomyces*. It shows a time lapse series of photographs of the growth of the *Phycomyces* sporangiophore (Cerda-Olmedo and Lipson, 1987). *Phycomyces* should bring us closer to understanding gravity at the molecular level. It is a beautiful organism that was studied by Max Delbrück and his group at the California Institute of Technology during the 1950s and 1960s. The sporangiophore is multinucleate, but it is just one cell.

Figure 3A (Cerda-Olmedo and Lipson, 1987) shows a normal and a mutant *Phycomyces* responding to gravity. The *Phycomyces* photoresponse is seen in Figure 3B (Cerda-Olmedo and Lipson, 1987). This is almost, but not quite, a one-quantum phenomenon, and the figure shows a young sporangiophore and a somewhat older sporangiophore responding to a weak unilateral light source. Owing to the efforts of Lipson and Dennison and others during the earlier work of the Delbrück group, there are multiple mutants available to show that the transduction apparatus, after signal reception, is common to all the responses. If one makes mutants one does not get the expected segregation of mutants insensitive either to gravity or to light. Rather, one mutation may eliminate phototropism, geotropism, carotenoid synthesis, and the avoidance response — all at one time, if the mutation occurs somewhere in the transduction sequence. This shows that many of the transduction response systems share common steps without regard to whether the initial stimulus is gravity or light.

Figure 3. (A) The gravitropic response of the *Phycomyces* sporangiophore as a function of time and taken at 0, 8, 11, and 13 min after the sporangiophore was placed in a horizontal position. (Photograph by H.H. Huenert. Reproduced, with permission, from Cerda-Olmedo, E. and Lipson, E.D., *Phycomyces*, Cold Spring Harbor Laboratory, 1987.) (B) The phototropic response of the *Phycomyces* sporangiophore as a function of time after initiating a unilateral light exposure. Photographs taken at 0, 2.5, 8, and 11.3 min after light exposure. (Reproduced, with permission, from Cerda-Olmedo, E. and Lipson, E.D., *Phycomyces*, Cold Spring Harbor Laboratory, 1987.)

Another phenomenon, although not depicted here, is a very unusual one (as though we don't have mystery enough!). *Phycomyces* shows an "avoidance response." Even in the dark, if one puts a glass rod near the sporangiophore, the sporangiophore will grow away from it. What the signal may be or what kind of sensing the organism is using is unknown. It avoids any object put into its path, even in darkness. My personal prediction is that the sporangiophore senses a perturbation of its bioelectric field by the rod.

*Phycomyces* does pose a problem similar to that discussed by Dr. Brown in Chapter 1 of this volume. *Phycomyces* does not have obvious statoliths, an observation that may provide us with an enormous clue. It does have organelles, and one could argue that they are heavy enough and large enough to settle or light enough to float. That remains an open question. However, the peculiar thing about *Phycomyces* is that it is a single cell, and yet it will bend toward the light. It must mean that the cell has done something to its cell wall on one side to make it more bendable, i.e., more extensible. So, the cell can selectively modify one wall or the other to evoke the tropic response. This is hard to understand simply in terms of the settling of a heavy body.

The force that causes this bending, once the wall is weakened, would be that studied by Van't Hoff many years ago. Solutes are present within the organism, and therefore the organism takes water from the external media. If the wall is weakened then it can expand and grow. We conclude that *Phycomyces* would be an ideal experimental organism for gravity-related studies.

STATOLITHS

Statolith is the name applied to dense bodies localized in special gravity-sensing cells called statocytes. Figure 4 is an example from Professor Sievers' research (Sievers and Schrotter, 1971) on growing tips of the alga *Chara*, and demonstrates that statoliths may be composed of inert material.

*Chara* has statoliths which are composed of barium sulfate, but statoliths in other organisms can also be composed of calcium carbonate or starch. This tells us that whatever the statolith is and whatever it does is independent of its chemical composition. It appears to depend on what the statolith falls against or through, because obviously the cell can accomplish with solid barium sulfate the same thing as with a well-organized starch-containing statolith. The variable composition of the statolith, which can be insoluble inorganic salts, seems to rule out postulates that the statolith itself does some chemistry.

Returning to higher plants, consider the work by my colleagues Somerville and Casper at Michigan State University and by Pickard at Washington University in St. Louis (Casper and Pickard, 1989), along with later studies at Ohio State University (Kiss et al., 1989). Figure 5A illustrates the growing root tip of a plant called *Arabidopsis*. *Arabidopsis* was referred to by Fritz Went many years ago as a botanical *Drosophila* because it can complete an entire life cycle in about two to five weeks. The figure shows a normal root tip of *Arabidopsis*, with well-defined statoliths. Casper and Somerville have produced a mutant of *Arabidopsis* that lacks phosphoglucomutase in the chloroplast (Figure 5B). Had it lacked phosphoglucomutase in the cytoplasm, it would be a dead *Arabidopsis* since phosphoglucomutase is essential for glycolysis. However, the deficiency was confined to the chloroplasts, preventing them from making starch, and thus there are no starch-containing statoliths. Nonetheless, this organism responds to gravity.

It has been shown that this mutant (Figure 5B) responds to gravity at a slower rate than does the wild type (Kiss et al., 1989). This was originally thought to be due to differences in growth rate between the mutant and wild type, but more recently it has been shown that the mutant responds more slowly to the gravity vector (Kiss et al., 1989). From this
series of findings, a very important conclusion can be drawn. A statolith, a heavy starch-containing statolith, can function as a rapid sensor of gravity that is highly directional. This mutant study is among the best evidence we have that statoliths can function as gravity directional sensors.

A second conclusion is also possible. A dense, starch-filled statolith is not required by all living systems to sense gravity. We conclude if a plant has a dense statolith, it can use the statolith as a gravity sensor that responds rapidly and with great directional accuracy. If, however, the plant lacks a statolith it can still sense gravity by other, possibly unknown, mechanisms. I think these are important conclusions. The following section will provide some evidence that these alternative sensors are bioelectric sensors.

First, a summary of some properties of statoliths. The statocyte usually contains one to eight grains of starch or some other inert material and is usually enclosed in a membrane. The statoliths should be about 1.5 times as dense as the cytoplasmic matrix in which they settle, and they should be about 1/10 to 1 micrometer in diameter. Mitochondria, plastids, nuclei, nucleoli, and the golgi all might serve, but as they become smaller and less dense there would be an increasing balance of their potential energy with the perturbations of $kT$.

These properties lead to an interesting suggestion for an experiment. The law of reciprocity, $gt = kl$, where $t$ = time in which $g$ is presented, $k$ = constant, and $I$ = intensity of response, should be studied in microgravity to determine how far the reciprocity principle can be pushed. If we can push reciprocity far enough by giving long exposures to fractional $g$ forces, and if the response does not deviate too far from the general rule of reciprocity, then I think that would rule out the settling of any dense body as a gravity sensor. At low $g$, $kT$ would become increasingly a problem and a point would be reached, e.g., at one-tenth $g$, in which no statolith would settle no matter how long the presentation time. I have strongly supported this experiment in the belief that it is a key to determining whether there are gravity sensors that do not involve the settling of a dense body in the cytoplasm. My reasons for questioning statoliths as the sole
gravity sensors include some of the above arguments and the fact that statoliths are not ubiquitous, and starchless mutants retain much of their gravity sensitivity. We must pursue this line of inquiry which points to gravity sensors that are not heavy falling bodies.

Another problem with statoliths is that they appear to be inert. The question arises — even when a statolith falls to the bottom of the cell, what does it do? That remains an important question. I have always liked to ask the question, is the falling apple the gravity sensing device of the apple tree? Newton could properly have made that conclusion. Will the reciprocity law hold at low g? We must know the answer to that question.

**ELECTROPHYSIOLOGY AND BIOELECTRIC PHENOMENA**

There are other kinds of stimulus sensing that may be related to gravity sensing, one of which is studied by electrophysiologists. My interest in electrophysiology dates back to student days in the 1940s. It was first awakened by the beautiful and classic work of Professor Lund at the University of Texas. Lund wrote a book entitled *Bioelectric Fields and Growth*, published in 1947. In that book Lund showed that following stimulation by light or gravity there were perturbations of the electric field of plants. He raised the question of whether the perturbation could, itself, be part of the transduction apparatus. I think it is, and today we are still struggling with this question.

Professor Andreas Sievers’ research shows that membrane depolarization occurs in a plant root within 8 seconds following a stimulus. Behrens et al. (1982) changed the orientation of the root from a normally vertical orientation to an inclined position about 45 degrees from the vertical and obtained a depolarization response, indicating that the plant had sensed gravity within 8 seconds. Similar studies were made by Tanada (1978a and b; Tanada and Johansen, 1980) within the last 15 years and with a somewhat similar time course. Tanada’s methods were not quite as elegant, and they extrapolate back to responses occurring in about a minute or two. Again, both the gravity and light stimulus cause a change in membrane polarization. Thus, I believe this response is ubiquitous.

Other examples of bioelectric phenomena can be informative. The examples in Figure 6 are from a chapter in a book by Weisenseel (1983), and they show a number of interesting things. In all cases the organism has a bioelectric current, and the changes that occur in the electrical field precede actual growth. The point where the current enters the cell, in the engineer’s sense, will be the point at which growth occurs. These conclusions appear to be universally true.

The first example shown in Figure 6 is that of the brown alga *Fucus*, and the electrophysiology was done by L. Jaffe. Also shown are lily pollen and barley root in experiments done by Weisenseel. The currents always appear before the growth phenomena are evident. Other species are the alga *Vaucheria* (experiments by Blatt and Kerser), the frog oocyte (studied by Jaffe), and regeneration of a newt limb.
In all cases depolarization occurred, followed by a current, and then by some manifestation of growth.

Many explanations have attempted to suggest how this occurs, but it is not yet clear. A partial explanation has been offered by Weisenseel. For a symmetric cell one might initially have a uniform distribution of ion pumps and ion leaks. The pumps are illustrated by little black circles in Figure 7, which illustrates the distribution of pumps and leaks in the unstimulated symmetric cell. Following a signal the pumps and the ion leaks redistribute themselves and become polarly arranged as indicated. Then a transcellular current begins to flow; it is small at first but can be amplified by means of ionic gradients. Finally, growth will occur with initiation occurring at the point where the current is entering the cell. This seems to be a good summary of the growth phenomena that are associated with a stimulus. The only thing I wish to add is that the bioelectric field itself may be the detector for the gravity stimulus.

THE CORN SEEDLING SYSTEM

This system is my specialty. Figure 8 shows a five-day-old corn (Zea mays) shoot that is growing in the dark. The pictures were taken at 15-minute intervals after the plant was moved from a vertical to a horizontal position (Bandurski et al., 1984). The system is very sensitive to gravity. Actually, the first signs of bending can be detected within about three to five minutes after the plant is moved from a vertical to a horizontal orientation. To the best of our knowledge each and every cell is responding, that is, a smooth continuous column of cells is bending. The question arises — if a single cell cannot feel gravity, then how does the whole organism feel gravity? It is the paradox of Zeno’s arrow! Our contention would be that each and every cell in this organism is sensing gravity, and each one is responding by an asymmetric growth.

To reiterate, the stimulus occurs, then membrane potential changes, and, finally, a bevy of chemical asymmetries develops. The one we have found most interesting is the asymmetric distribution of the plant growth hormone indole-3-acetic acid (IAA) (Bandurski et al., 1990). Another chemical asymmetry that occurs in a similar time span is that of calcium (Slocum and Roux, 1983). These two things (IAA and calcium) appear to respond very quickly to membrane depolarization. Then, after longer times asymmetries develop with regard to potassium. Even sugar ultimately becomes asymmetrically distributed (Momonoki, 1988).

An argument can be made that the study of chemical asymmetries and how they arise may be fruitful, because it does not deal with the enormous complexity of growth. The question can be simply — how does the organism get more
of something onto one side than the other? That question may be a little easier to approach than the many complexities of growth.

The corn shoot system has an advantage for such studies because the experimental part of the plant called the mesocotyl is a region from the seed up to the first node that has only one central stele. All of the nutrients come to the shoot through the stelae and then move from the stelae outwardly into the cortex. They can be gated out from the stelae into the surrounding cortical tissues. A diagram of the relationship between the stelae and cortex is seen in Figure 9. Within the stelae there are different kinds of vascular tissues, cells that are specifically designed to conduct water, salts, and organic nutrients. The cortex itself is a hollow cylinder which sheaths the stelae, and it, of course, can receive nutrients only from the stelae.

We have shown that an asymmetrical hormone distribution can occur within three minutes following the gravity stimulus. An equally important discovery is that both free and ester IAA, i.e., total IAA, increase on the lower side of the shoot (Bandurski et al., 1990). This means that transport must be involved since no amount of interconversion of free IAA to ester or ester to free IAA will increase the total IAA on one side of the plant—since no de novo synthesis occurs (Bandurski et al., 1990).

Table 1 shows the results of these IAA measurements (Bandurski et al., 1990). Each analysis is done by gas chromatography-mass spectrometry using an internal standard. The concentration of the hormone is \( 10^{-7} \) M in the tissue, so considerable sensitivity is required.

From Roux's data (Slocum and Roux, 1983) calcium asymmetry is also shown to develop within minutes after the gravitational stimulus. Interestingly, the hormone indoleacetic acid, an anion, its ester, a neutral compound, and calcium, a cation, are all responding to the gravitational stimulus. So, a negatively charged compound, a neutral compound, and a

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Figure 8. Gravity response as a function of time for a five-day old seedling of *Zea mays*. Photography was by means of a phototropically inactive green light at 15 min intervals by Dr. P. Dayanandan. (Reproduced, with permission, from Bandurski, R.S., Schulze, A., Dayanandan, P., and Kaufman, P.B., *Plant Physiology* 74: 284-288, 1984.)

Figure 9. A diagrammatic representation of a seedling of *Zea mays* indicating how voltage gating might regulate the selective movement of the plant hormone precursor indole-3-acetyl-myoo-inositol (IAI nos) and/or its hydrolysis product, the active hormone, indole-3-acetic acid (IAA) from the vascular tissues of the stelae into the target tissues of the cortex-epidermis. (Reprinted, with permission, from *Advances in Space Research*, in press, Bandurski, R.S., Schulze, A., Jensen, P., Desrosiers, M., Epel, B., and Kowalczyk, S., The mechanism by which an asymmetric distribution of plant growth hormone is attained, 1991.)
Figure 10. A transmission electron micrograph of *Zea mays* showing the plasmodesmata that connect the tissues of the vascular stele to the surrounding cortical cells. Experiments with polar dyes indicate that plasmodesmata provide the only connection between cells of the stele and cells of the cortex. (Unpublished photograph of Dr. Robert Warmbrodt, USDA.)

Table I. Percent of Free and Total (Free Plus Ester) IAA in the Lower Half of the Mesocotyl Cortex of a *Zea mays* Seedling as a Function of Time After Moving the Seedling from a Vertical to a Horizontal Position. (Bandurski et al., 1990)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Free IAA (%)</th>
<th>Total IAA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>50 ± 0</td>
<td>50 ± 0</td>
</tr>
<tr>
<td>1</td>
<td>50 ± 0.1</td>
<td>50 ± 0.1</td>
</tr>
<tr>
<td>3</td>
<td>56 ± 3</td>
<td>60 ± 1</td>
</tr>
<tr>
<td>5</td>
<td>55 ± 3</td>
<td>59 ± 1</td>
</tr>
<tr>
<td>15</td>
<td>56 ± 1</td>
<td>62 ± 5</td>
</tr>
<tr>
<td>30</td>
<td>56 ± 2</td>
<td>56 ± 1</td>
</tr>
<tr>
<td>90</td>
<td>57 ± 3</td>
<td>54 ± 2</td>
</tr>
</tbody>
</table>

Table II. Movement of 5-[^3]H]-IAA from Stele to Cortex during Gravity Stimulation. Radioactivity was administered by endosperm injection. (Bandurski et al., 1990)

<table>
<thead>
<tr>
<th>Loading Period</th>
<th>% Total Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-min load</td>
<td></td>
</tr>
<tr>
<td>Cortex, left half</td>
<td>23.1 ± 1.8</td>
</tr>
<tr>
<td>Cortex, right half</td>
<td>23.7 ± 2.6</td>
</tr>
<tr>
<td>Stele</td>
<td>53.2 ± 3.8</td>
</tr>
<tr>
<td>30-min load, followed by a 90-min gravity stimulus*</td>
<td></td>
</tr>
<tr>
<td>Cortex, upper half</td>
<td>28.5 ± 1.2</td>
</tr>
<tr>
<td>Cortex, lower half</td>
<td>35.9 ± 1.8</td>
</tr>
<tr>
<td>Stele</td>
<td>35.6 ± 1.7</td>
</tr>
</tbody>
</table>

* Plants were kept in a vertical position for a 30-min loading period following injection. Plants not sacrificed were then given a 90-min gravity stimulus by placing the plants in a horizontal position.
Figure 11. A diagrammatic representation of how ligand binding or changes in electrical potential might gate the movement of solutes from cell to cell. (Reproduced, with permission, from Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J.D., Molecular Biology of the Cell, Garland Publishing Inc., 1983.)

positively charged compound all become asymmetrically distributed. This convinces me that asymmetry is a matter of gating, because if it is due to specific carriers, one would need bevies of specific carriers all similarly affected by the stimulus. One way to interpret the data is that, by some mechanism, the organism can open and close gates that connect the stele to the surrounding cortical tissues (see Figure 9).

The experimental results that really led us to the idea of gating are given in Table II. For these experiments the plant was labelled with radioactive IAA in the seed while the plant was still in the vertical position. The radioactive hormone ascended the central stele into the young shoot. Next, the seed, which was the source of the radioactivity, was removed, and the plant was held horizontally to reorient the gravity stimulus. The bottom half of the cortex, which will grow faster, equilibrates with the stele, whereas the upper half of the cortex, the part that will not grow, does not equilibrate with the stele. It is this experiment that first convinced us that some kind of gating between stele and cortex was involved. The gravity stimulus must have opened the gates in the channels between stele and cortex on the lower side of the horizontal stem and closed the gates in the channels between the upper side of the stele and cortex.

In collaboration with Dr. Warmbrodt at the U.S. Department of Agriculture we obtained some pictures of the plasmodesmata, the channels that connect the stele to the cortex (Figure 10). There is no apoplastic communication between the vascular tissue and the cells in the cortex. Anything that moves from the stele must pass through the small plasmodesmata. The plasmodesmata are large relative to an ion channel or to a gap junction. At least we have been able to peel off that much of the skin of the banana, and we are looking at a target for gravity — the plasmodesmata. Incidentally, the whole stele is encased in a Bakelite-like layer, a lignified and suberized sheath. Things that move from stele to cortex must move through these structures. We need to examine them to find out how the channels are gated and what kind of channels they are. If these channels are indeed involved in gravitational responses, we have made a genuine reductionist advance and can focus on this relatively small piece of the problem.

One can envisage the membrane as an ocean of lipid, with icebergs of protein floating in it. If there are proton
motive voltage sensors in these proteins that can change the conformation of the protein, they can gate whatever will go through. Selective filters can determine which ion or which organic compound is presented and the gate can then open or close by means of alteration of the potential across the membrane. Another variant of this picture is found in the very beautiful textbook by Alberts et al. (1983) (Figure 11), illustrating something of what is known about voltage and ligand gating of pores.

In our laboratory we examine the effects of applied voltages upon the movement of compounds within the plant. We apply 5 volts across 8 to 10 cm of plant tissue; however, the voltage drop across a 10-micrometer cell is less than a millivolt per cell, in fact, 0.6 mV per cell — not a huge potential. Yet their effects are extremely dramatic, as Dr. Desrosiers’ research has shown in Figure 12, which illustrates the effect of an applied voltage on growth. If one makes the tip of the plant negative there is no effect on growth or only a slight stimulation. If one makes the tip of the plant positive, growth is essentially stopped — a 90% inhibition. The same current is flowing at either polarity; obviously, there is something special about this small controlling voltage (Desrosiers and Bandurski, 1988).

We have sought an explanation and have found two chemical responses that result from making the tip of the plant positive (unpublished). One is that the ester of indole-3-acetic acid (IAA) becomes locked in the stele and does not move out. The second is a reduction of the rate of calcium movement from seed up and into the shoot. So, this applied voltage does seem to be targeting at least two materials. We hope, with subsequent work, to account for the rate of decrease of growth in terms of some chemical entity.

Another version of gating in which substances need not move from cell to cell is shown in Figure 13. Within the cell...
itself one could encounter large electrophysiological changes. Visualize the cell as being a whole series of microtubules and through these tubules compounds are flowing that can be oxidized and reduced (Bandurski et al., 1986). If anything is done to interfere with the rate at which a reductant flows to the cathode by varying the pore size of these microtubules, there will be a change in the amount of external current that flows. Thus, pressure on one side of a cell, caused by the weight of the tissue, can be transduced into a current flow with the resultant growth such as shown in Figure 6. Our research presumes that events like this occur within the cell, and that a changing potential could significantly alter the cell's metabolism.

CHEMOTAXIS

I wish to briefly comment on chemotaxis. Figure 14 is an original drawing of an experiment by Pfeffer adapted from Koshland (1980). He simply introduced a capillary filled with a chemical attractant into a suspension of bacteria. The bacteria swam toward the attractant as illustrated and accumulated in the capillary. In the case of bacterial chemotaxis, an attractant binds to a receptor on the exterior of the cell, e.g., a receptor for aspartate or serine binding on

Figure 14. A diagram of the original Pfeffer experiment showing the concentration of motile bacteria into a pipette containing a chemical attractant such as an amino acid. (Reproduced, with permission, from Koshland, D.E., Jr., Bacterial Chemotaxis as a Model Behavioral System, Raven Press, 1980, p. 6.)

Figure 15. A diagrammatic sketch indicating how a proton motive force may be generated by cell metabolism and then used to power a system of bacterial cell motility. (Reproduced, with permission, from the Annual Review of Microbiology, Volume 37, ©1983 by Annual Reviews, Inc.)
the membrane. The binding of the attractant to the receptor then causes a change in proton motive force (PMF) which is manifested by a change in rate of the beating of the cilia. Barry Taylor and others have shown that you can actually vary this rate by changing the PMF across the membrane (Figure 15). So, there appears to be a direct coupling between the PMF and the beating of the cilia (Taylor, 1983). Another example is the more familiar system of light perception in phototaxis. The light is received and, depending upon whether the organism is a vertebrate or an invertebrate, the stimulus can open or close sodium channels, and the signal is thus amplified.

**SUMMARY**

What, therefore, are the possible linkages between gravity detection and transduction? First, of course, it may be that gravity causes stratification of dense bodies within the cell (Sievers and Hensel, 1982). The resultant changes in concentration could induce changes in hormones or second messengers that control asymmetric growth. A second possibility is that gravity causes stratification of dense charged bodies within the cell. The movement of the charged body through the cell's bioelectric field may open and close hormone and transport channels and, again, asymmetric growth could result (Bandurski et al., 1990). Third, gravity-induced hydrostatic pressure could cause deformation of the cell's fine structure, and that perturbation may ultimately result in asymmetric growth of the cell line (Bandurski et al., 1986).

Gravitational biologists should consider the possibility of a direct coupling between the very weak physical force of gravity and the complex electrical circuitry within the quasi-crystalline matrix of the cell. Such coupling would be difficult owing to the disparity in strength between the weak force of gravity and the strong force required for cellular changes. Nonetheless, the cell possesses strong amplifying capabilities and the possibility of coupling should not be ignored. This is a difficult problem, but a fascinating one, and if we persevere, we can solve the problem.

**REFERENCES**


