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Perception of Gravity by Plants

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I. OBJECTIVES

An environmental cue widely used by plants to guide development is gravity. Although many things are known about plant responses to gravity, one fundamental aspect remains obscure, i.e. the means by which the *physical* stimulus of gravity is transduced into a *physiological* response which the plant can use to guide development.

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In order to analyse that transduction, both the physics and physiology must be considered. Although the physical aspects have been considered before, notably by Audus (1979), some reiteration and expansion of earlier discussions is useful to interpret recent data and to indicate valuable concepts to pursue. In this chapter the intention is to give these physical laws deeper attention than they have received in the past. This discussion of physics and gravity perception should point out paradigms, some provocative, which will be helpful in developing new hypotheses of gravity perception. The physical behaviour of objects on a human scale is very different from that on the subcellular scale. It is easy, when imagining how gravity perception might work, to make erroneous assumptions about how various components will act. It is hoped to give the reader an intuitive grasp of how gravity acts on plant cells and how that may be turned into physiological information.

The perception of other physical stimuli, light and sound, has recently been well characterized in animals. Metabolic syndromes in these transduction mechanisms may have parallels in gravity perception by plants. The experimental approaches which led to the elucidation of the animal transducers also provide a useful guide to promising future approaches.

Gravitropism is the result of a series of events, and the terms used for each step are as defined by Hensel (1986a). *Susception* is the initial physical reaction by a mass in the gravitational field. *Perception* is the conversion of the physical signal to a physiological one. *Transmission* is how the signal moves from the cells where gravity is sensed to those where growth occurs. The *response* is the differential growth which results in curvature. The term transduction is sometimes used to describe the step called perception, but here a more specific meaning will be used: the carrying over of energy or information from one form (or place) to another. By that definition, transduction occurs in each of the four steps of gravitropism. Gravity sensing (or gravisensing) is also a common term used to describe part of gravitropism, usually corresponding to susception and perception. There is not yet a consensus on the terminology describing the steps of gravitropism; as these steps become physiologically better defined, so will the words used to describe them.

In order to discuss potential mechanisms of perception, gravity will first be considered from a physical perspective and related to a plant's susception of gravity. Second, the types of signals which may be involved in transmission will be reviewed. In the main section, the means by which the information provided by susception can be transduced to the kinds of signals which may be transmitted will be considered. Some potential perception mechanisms will be evaluated in terms of their likelihood of performing as rapidly and as sensitively as does the true gravity-sensing system.

II. SUSCEPTION

The first step in gravitropism is a physical action of gravity on some element in the plant, which is called susception. Gravity is an attractive force on a mass; it is that force which a graviresponding plant must use to orient itself relative to the gravitational field. Although gravity acts on every atom in the plant, the number of relevant interactions is limited. This section will cover the action of the gravitational force and limitations to its detection.

A. HOW GRAVITY ACTS

To analyse how a plant senses gravity one must know how gravity interacts with physical objects. Newton's law of gravitation holds that two objects attract each other with a force proportional to the product of their masses and inversely proportional to the square of the distance between them:

$$F_g = Gmm'/r^2$$

where m and m' are the two masses and r the distance between them. G is the experimentally determined gravitational constant. The attractive gravitational force will tend to move two particles towards each other, which we can observe, for example, as the attraction between an apple and the earth.

In the case of gravity sensing by plants on the earth's surface, one particle is the earth, and the other is within the plant. The distance between the particles is the radius of the earth because the gravitational force of a sphere acts as if all the mass is at its centre. Thus the values for G, m and r are constant and the gravitational equation reduces to: $F_g = (9.8 \text{ m s}^{-2})\ m'$. The gravitational force then depends on the mass (m') of whatever particle we are considering.

A plant must sense gravity by detecting the attraction between the earth and the mass of some object associated with the plant, which will be referred to as the sensing particle. For that attraction to be detectable, the sensing particle must do work (in the thermodynamic sense) on something to cause a change in the physiological activity of the plant. Displacement of the particle in the gravitational field is required to convert gravitational potential energy to work. If the particle does not move, there is no energy to alter the physiology of the plant.

A simple example of work, as defined in physics, is a mass moving against gravity. The work done is the force applied ($g \times m'$) times the distance the mass is moved. This can be illustrated by a seesaw (Fig. 1). With mass A on the low side, if a larger mass, B, is placed on the high side it will exert more force and drop. As it drops, it loses gravitational potential energy and does work on mass A. The work done on mass A causes the

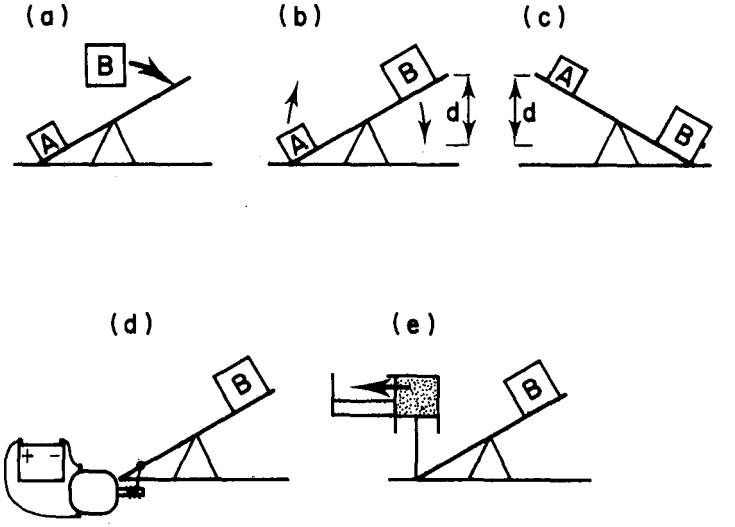

Fig. 1. Displacement of a mass in a gravitational field does work. (a) Mass A is at rest on a seesaw; a larger mass (B) is placed on the high side of the seesaw. (b) Mass B exerts more force and therefore moves the seesaw. As it descends, it loses potential energy ($B \times g \times d$) and does work on mass A by raising it in the gravitational field. (c) The mechanical work done on mass A increases its gravitational potential energy by $A \times g \times d$. The work could also be converted to: (d) electrical energy by running a generator; or (e) chemical energy by running a reverse osmosis unit which separates solutes from water.

mass to rise, increasing its gravitational potential energy. The rising arm of the lever could also cause a generator to turn, creating electrical energy; or it could push the piston in a reverse osmosis unit, creating chemical potential energy by purifying water. Work can convert the gravitational potential energy to many other forms of energy, depending on the transduction mechanism. Therefore, a simple model of susception can lead to many possibilities for perception.

Susception can occur in a number of possible ways: the sensor may do work either by being denser than the surrounding medium and sinking, or by being lighter and rising. The sensor may be inside or outside the cell. There are many objects within the cell which may move relative to each other due to their differing densities. The cell could perceive motion, displacement or position of the sensor. We will see what evidence there is for each of these things happening.

B. THERMAL MOTION

The mass acted on by gravity is also being constantly agitated by collision with water and other molecules which have kinetic energy due to heat.

This random thermal motion is commonly seen as Brownian motion. For the gravity sensor to be effective, it must be relatively insensitive to the random thermal energy but be very sensitive to changes in the direction of gravity. An important limiting factor for a sensing mechanism, then, is thermal noise.

The magnitude of thermal energy on a particle is $\frac{1}{2}kT$ in each dimension, where k is Boltzman's constant (1.38×10^{-23} J K^{-1}) and T is the absolute temperature. At room temperature, $\frac{1}{2}kT = 2 \times 10^{-21}$ J. The thermal agitation of a particle is independent of the particle's mass, so the effect of gravity relative to the thermal noise is greater the more massive the particle. Thermal noise sets one lower limit on the minimum work which must be done by a sensor during susception.

The rate of a chemical reaction is limited by the activation energy of the reaction. Thermal motion of the reactant provides the energy needed to overcome the activation energy of a chemical reaction. For a reaction caused by a mechanical stimulus, in contrast, the sensor should be selectively activated by the stimulus rather than thermal motion. This can be accomplished if the activating reaction has an activation energy high enough that it is rarely stimulated spontaneously. If the activation energy is high, the minimum stimulus must be correspondingly large. There is a trade-off between a sensor's sensitivity and its selectivity.

The effect of activation energy on the spontaneous reaction rate can be calculated (Fig. 2). The frequency of activation by thermal energy decreases very rapidly as the activation energy increases. The figure is based on the Arrhenius equation: Rate $= A\, e^{-E/RT}$. The light sensor in vision is rhodopsin, which is physically activated by the energy in photons. Rhodopsin is stimulated only very rarely by heat. If the enzyme reduced the activation energy for the reaction only two-fold, thermal activation could occur 10^{10} times faster. Rhodopsin functions well in light perception because its activation energy is high enough to make spontaneous triggering very rare (about once every 1000 years per molecule), but is low enough that the light stimulus contains ample energy.

By a similar approach, this figure can be used to estimate the activation energy of gravity perception. The rate of the first step in gravity perception by the thermal motion of the suscepting body must be much less than the rate during gravistimulation. The activation energy of that step must therefore be high enough that the thermal energy ($\frac{1}{2}kT$) produces 10^{-3} to 10^{-4} activations for each one produced by a small gravistimulation. From Fig. 2 it can be determined that the activation energy of the first step of perception which fits this criterion is 3–4 $\times$ 10^{-20} J. Thus the activation energy, and hence the amount of work required during susception, can be fairly precisely estimated. Presumably there would be many activating events per cell per second, each using about 4×10^{-20} J. This estimate applies regardless of the specific mechanisms of susception and perception.

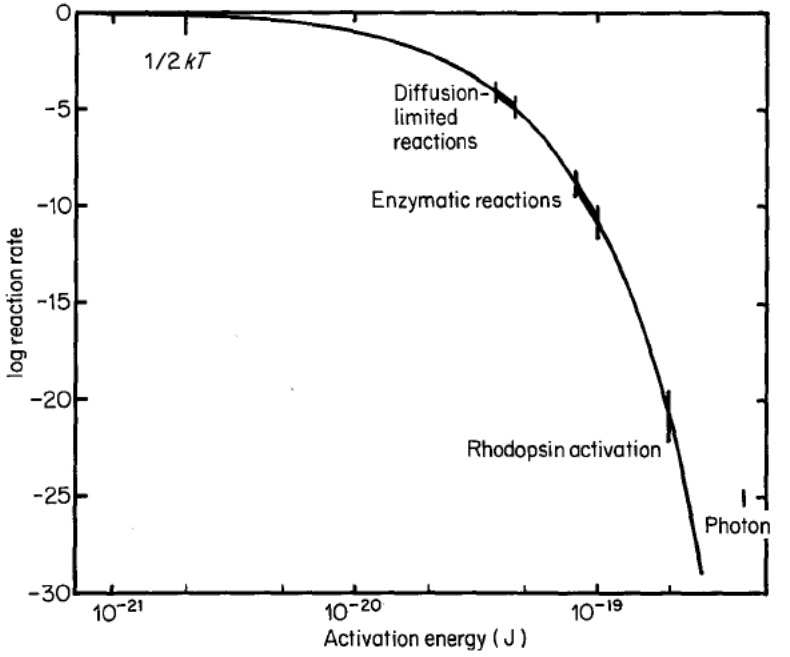

Fig. 2. The effect of activation energy on the relative rate of reaction. Enzymic reactions have activation energies clustered over a small range, yet one where the rate of a reaction can vary by many orders of magnitude. Spontaneous thermal activation of the light-sensing molecule rhodopsin occurs at a very low rate. A gravity sensor must be insensitive to thermal energy ($\frac{1}{2}kT$), so its activation energy would be expected to be 3–4 $\times$ 10^{-20} J. Intermediate marks on the abscissa are 2 and 5 times the order of magnitude. The formula for this curve is: $\log R = -E_A/kT$.

Although small effects of gravity can be amplified through various biochemical cascades, these amplifiers will not discriminate between the desired signal and noise. This lack of discrimination is why amplification alone does not provide the necessary sensitivity to detect weak stimuli. For an amplifier to be useful, the total signal must be filtered or averaged using some criterion to reduce the noise. The possibilities for such processing by the perceiving system will be discussed in a later section.

The only measurable physiological response of a plant which yields information about susception is the presentation time, which is the threshold gravistimulation required to elicit a growth response. There is a reciprocity between the presentation time and acceleration when the acceleration is changed from $1 \times g$ by centrifuging or clinostatting (Johnsson, 1965). A metabolic process would not exhibit this sensitivity to the gravitational force, so the presentation time must reflect the physical process of susception. The presentation time may also include some time

required for the physiological steps of perception (Johnsson, 1965). The inverse relationship between the force and the presentation time is consistent with a threshold displacement of a sensor required to stimulate perception. This threshold may be thermal noise.

Hair cells in the cochlea (the auditory receptor in animals) can detect extremely small stimuli through tuning and time-averaging the repetitive stimulus of a sound wave. The limit of perception corresponds to a motion of atomic dimensions (Harris, 1967), even though sensitivity is limited by thermal motion.

A measure of the ability of the sensor in susception to overcome thermal noise is the minimum amount of stimulation which will produce a gravitropic response. *Avena* roots respond to $3 \times 10^{-4}g$ when stimulated as long as 68 h on a clinostat (Shen-Miller *et al.*, 1968). In lettuce seedlings grown in centrifuges aboard the Salyut 7 space station, the shoots had a threshold response at $3 \times 10^{-3}g$, and the roots at much lower gravity (Merkis *et al.*, 1985). At $1 \times g$ the presentation time can be as short as 7 s for *Lepidium* roots (Larsen, 1969). Such high sensitivity can be achieved only by signal averaging and with a substantial responding mass.

The potential sensors in a plant are limited to those that are large enough to move a perceptible amount relative to thermal noise within the time it takes a plant to detect a gravistimulus. That much motion must be produced even by the very small stimuli to which plants are capable of responding.

III. TRANSMISSION

Gravity sensing occurs only in certain regions of the plant, but gravitropic curvature rarely occurs in those cells which sense gravity. A signal which indicates how the responding cells must alter their growth passes from one group of cells to the other. Transmission has been excellently reviewed by Audus (1979). In roots, for example, gravity is sensed in the root cap, but growth occurs in the elongating zone of the root apex, several millimetres away (Darwin, 1899). In etiolated beans, gravity is sensed in the cotyledonary hook, but curvature occurs 2–3 cm lower in the stem (Hart and Macdonald, 1984; Verbelen *et al.*, 1985). The sensing and responding cells may also be separated radially. In coleoptiles, gravity is sensed in the inner mesophyll, but growth is controlled by cells in and near the epidermis (Thimann and Schneider, 1938; Kutschera *et al.*, 1987). Curvature can only be expressed in cells which are growing or which may be induced to grow; sensing cells need not be near a growing zone. A signal must therefore travel from one group of cells where gravity is sensed to another where growth is controlled. The signal may move symplasmically or apoplasmically; in either case the signal must leave the cell where it originates by crossing the plasma membrane.

Membrane transport is tightly regulated, and therefore is a good place to focus attention when trying to narrow down the type of signal which is elicited from the sensing cell. In roots, transmission of the gravistimulus appears to be through modulation of a growth-retarding factor in the elongating zone (Shaw and Wilkins, 1973), potentially an inorganic ion rather than a plant growth regulator (Mertens and Weiler, 1983). In the shoot it is likely to be modulation of a growth-stimulating factor (Dolk, 1933). In grass nodes, a growth initator moves to the epidermis (Kaufman and Dayanadan, 1984). Perception therefore occurs by a mechanism which causes this kind of modulation. Below, the biological mechanisms which elicit such signals are discussed and those which promise to be relevant to gravity perception are identified.

Intercellular communication can be either electrical or chemical. The most dramatic example of bioelectrical communication is the nervous system of animals. Chemical communication is typified by hormones, second messengers and plant growth regulators. The nature of the transmitted signal indicates the kind of reaction which is the final step of perception.

A. ELECTRICAL TRANSMISSION

Signals are often transmitted electrically, both in biological and engineered systems. A biological electrical signal can have many manifestations: an action potential, an electrical gradient or electrophoresis in an electrical field. Each of these manifestations could transmit a physiological signal.

1. Action Potentials

Action potentials allow rapid communication. The signal for leaf folding in *Mimosa pudica* (sensitive plant) and *Dionea muscipula* (Venus flytrap) are carried by action potentials (Pickard, 1973a). In *Mimosa*, vibration causes an action potential which is transmitted to pulvini at the base of petioles. There, the action potential triggers ion fluxes which cause turgor changes, folding the leaf. In *Dionea*, stimulation of a trigger hair sends an action potential to the leaf base (Burdon-Sanderson, 1873; Benolken and Jacobson, 1970), initiating rapid growth (Williams and Bennett, 1982).

Action potentials are rapid and transient changes in the membrane potential in response to a stimulus. The change in potential is due to increased permeability of an ion which is far from its equilibrium and which normally has a low permeability. The membrane potential then approaches the equilibrium potential for that ion. The potential difference between a stimulated cell and its neighbour is detected, presumably across plasmadesmata, and triggers an action potential in the second cell. This process continues down the line of excitable cells. Cells which can transmit an action potential are termed excitable because they respond actively to a stimulus.

Action potentials have been measured in many plants, especially in association with rapid movements including growth (Pickard, 1973a), but there are no examples analogous to the processes in gravitropism. Apparent action potentials have been noted in gravitropic *Allium* roots (Berry and Hoyt, 1943) but not in *Lepidium* roots (Behrens and Gradmann, 1985) Action potentials remain a possibility for transmission of the gravitropic stimulus, but evidence to support this possibility is lacking.

2. Electrical Gradient

A potential applied apoplasmically across a tissue can affect growth (Schrank, 1948; Evers and Lund, 1947; Moore *et al.*, 1987). Gravistimulation does produce an electrical gradient (Schrank, 1947; Grahm and Hertz, 1964; Tanada and Vinten-Johansen, 1980; Behrens *et al.*, 1982; Björkman and Leopold, 1987a), raising the possibility that curvature is the result of an electrical field. Although ion currents flowing in such an electrical field gradient have been proposed as guides to development in many tissues (Jaffe and Nuccitelli, 1974), there is no evidence that the field is sensed directly. A direct sensor would not be unheard of: magnetosensors, which sense magnetic fields, are known in biology (Lohmann and Willows, 1987).

3. Electrophoresis

An electrical field applies an attractive force to charged particles; therefore, an apoplasmic electrical gradient across a tissue causes mobile ions to move across the tissue through the cell walls. The ions, redistributed by electrophoresis, may then influence growth or act allosterically to modulate growth regulators (Hasenstein and Evans, 1986).

There is evidence against electrophoretic translocation of Ca^{2+} in the establishment of tissue asymmetry, and it is applicable to other ions as well. Curvature is towards the positive pole of an applied potential gradient in both positively gravitropic roots (Björkman, 1987) and negatively gravitropic shoots and coleoptiles (Schrank, 1948; Woodcock and Wilkins, 1969a). Transverse movement of Ca^{2+} is towards the slower-growing, positively charged side both in roots (Lee *et al.*, 1983) and in coleoptiles (Slocum and Roux, 1983). Calcium ions are therefore moving against the electrical gradient; they are not being electrophoresed. Auxin anions would electrophorese towards the slower-growing side in both cases. That is compatible with its role as a growth inhibitor in roots, but there is apparently no auxin redistribution in roots (Mertens and Weiler, 1983). In shoots, auxin stimulates growth and is redistributed against its electrical gradient (Mertens and Weiler, 1983; Bandurski *et al.*, 1984). Auxin would perhaps not be susceptible to apoplasmic electrophoresis in any case because at the low wall pH it would be in the uncharged, protonated form. Thus, existing data disprove such simple models of growth modulation through electrophoresis

of biologically active ions. Nevertheless, electrical polarization appears to be a common feature of the gravitropic response.

An electrical gradient is established by differences in electrogenic ion transport across the plasma membrane. Electrogenic transport occurs when a charge is moved across a membrane without a balancing charge, resulting in a change in the electrical potential across the membrane. The electrogenic transport could be varied by changing the activity of an electrogenically ion-transporting ATPase, such as the proton pump. The membrane potential could either hyperpolarize or depolarize, depending on whether the activity of the pump were increased or decreased. Depolarization could also be effected by increasing the permeability to some ion which normally has a large electrochemical gradient across the plasmalemma, as in action potentials. Such a permeability increase would most likely result from opening of a specific ion channel and ion movement through it down the electrochemical gradient without balancing counterions. Regulation of ion transport is an area of active research, and there are experimental means of determining how electrical effects of gravistimulation are generated.

B. CHEMICAL TRANSMISSION

The unequal signal to the growth zone may be generated by direct chemical transport, with the electrical polarization which results from ion movement being incidental. Signal substances transmitted by this type of mechanism would be those which are actively transported by the cells using polarly distributed carriers for directional facilitated diffusion, or even specific ATP-driven transport proteins.

1. Ion Pumping

Directional active transport of ions is widespread in plants to serve their mineral nutrition needs. The systems which are in place to distribute mineral ions in the plant may be adapted to transmit information, or similar independent transport systems could be dedicated to this role. Applied Ca^{2+} can cause curvature in corn roots, even if they are decapped (Lee *et al.*, 1983), suggesting that an apoplasmic Ca^{2+} gradient may be the transmitted signal. The increased apoplasmic Ca^{2+} concentration may sensitize the tissue to auxin which is present in growth-inhibiting concentrations (Hasenstein and Evans, 1986; Salisbury *et al.*, 1985). It may also act directly on the wall to inhibit growth (Cleland and Rayle, 1977). Apoplasmic Ca^{2+} ions therefore fulfil the requirements of a signal substance.

Transverse Ca^{2+} fluxes have been measured (Lee *et al.*, 1983) and wall calcium has been shown to redistribute (Slocum and Roux, 1983) following gravistimulation. The redistribution of only one other inorganic ion, K^+, has been measured. Although K^+ redistribution is important in pulvinus move

ment in seismonastic *Mimosa*, it does not generate the response of the pulvini to gravistimulation (Roblin and Fleurat-Lessard, 1987).

If Ca^{2+} ions are electrogenically pumped towards the slower-growing side of a tissue, that side will become electropositive relative to the faster-growing side. Such polarization has been notoriously difficult to measure (Woodcock and Wilkins, 1969b). Reliable measurements in coleoptiles show that polarization is a consequence, not a cause, of auxin redistribution (Grahm, 1964). Apparent upward currents in root caps following gravistimulation (Behrens *et al.*, 1982; Björkman and Leopold, 1987a) imply that the upper side is positive relative to the lower. This would be consistent with electrogenic cation transport towards the upper side. However, Ca^{2+} move towards the lower side of root tips (Lee *et al.*, 1983). A calcium-transporting ATPase has been discovered on the plasma membrane (Rasi-Caldogno *et al.*, 1987), but the effect on the charge distribution is unclear because the Ca^{2+} are exchanged for protons in an unknown stoichiometry. Unfortunately, there is not quite enough information available to determine whether direct pumping of an ion may be the transmitter of the gravity signal.

2. Growth Regulator Pumping

Polar (or directional) transport of growth-regulating substances would more directly effect differential growth than ionic messengers. Evidence is weak for growth regulator redistribution in roots (Mertens and Weiler, 1983; Jackson and Barlow, 1981), but good for above-ground parts (Pickard, 1985) with some exceptions (Trewavas, 1981).

Polar transport of auxin occurs through an auxin-translocating carrier in the plasma membrane, down an electrochemical gradient maintained through protonation of indoleacetate with protons supplied by the plasma membrane proton pump (Rubery and Sheldrake, 1974). The net result is ATP-driven auxin transport. The transport is polar because the IAA carrier is located only on one flank of the cell. This transport requires Ca^{2+} transport from the auxin sink (Niedergang-Kamien and Leopold, 1957), though the biochemical basis for this requirement is unknown.

A gradient across the tissue may be established through two kinds of cellular response. Either the top and bottom of each sensing cell respond differently, with each sensing cell behaving similarly, or the cells in the upper part of the tissue may act differently from those in the lower part. The membrane potential of sensing cells in *Lepidium* root caps respond to gravistimulation; those on the lower side hyperpolarize slowly whereas those on the upper side depolarize rapidly yet transiently (Behrens *et al.*, 1985). This is a clear example of different responses by cells in different parts of the sensitive region. This result is expected if the cell is organized so that the flank towards the epidermis differs from the side towards the centre of the tissue (Volkmann and Sievers, 1979). In grass nodes, such an organiza-

tion is believed to induce auxin transport only from cells on the lower part of a horizontal node (Wright and Osborne, 1977).

Whether an electrical gradient is established through change in electrogenic ion transport or a chemical gradient is established by polar transport of some substance, modulation of transport across the plasma membrane is necessary. The tissue gradient can be established either by asymmetric transport across each sensing cell or by cells on the bottom of the tissue responding differently from those on top. Because there is intercellular signalling, in all cases a change in plasma membrane transport would be involved. Based on presently available data we cannot definitely eliminate any of the transmission methods: ion channels, plasmadesmata, proton pump modulation, action potentials, polar transport of ions or growth regulators, and activation of a specific porter.

IV. PERCEPTION

The section on susception described ways in which perception could be triggered. The section on transmission described the type of signal produced by perception. Taking the inferences about perception from those sections into account, this section will establish how perception is likely to work and will consider some mechanisms which may safely be rejected.

The mass with which gravity interacts in susception must not only detect gravity, but must cause some physiological response. The effect of perception is to transmit a polar signal across the plasma membrane of some or all of the cells in the sensing tissue. The transduction from an intracellular stimulus to an extracellular signal, perception, could occur through a direct interaction between the sensing mass and a cell component which elicits the transmitted signal. More likely, it is mediated through a cascade of intracellular messengers to trigger transmission of a signal. An intermediate reaction cascade amplifies the signal and provides an opportunity for modulation of the message by other cellular processes.

To consider the ways susception could produce a transmitted signal, biological signal transduction in general will be discussed, and then compared to what we know about gravity perception. Some hypotheses which have been proposed for gravity perception in plants will also be discussed.

A. SIGNAL TRANSDUCTION OVERVIEW

As a basis for discussing the mechanism of gravity perception in plants, it is worthwhile to first review signal transduction in some sensory systems which are better understood. There may be analogous patterns among the sensory systems which can be useful in predicting perception of gravity by plants.

1. Other Sensory Systems

The photoreceptors in eyes of invertebrate animals are probably the sensory system which is best characterized at the physiological level (Steive, 1986). Perception of light begins with the absorption of photons by rhodopsin in the plasma membrane of retinal rod cells. Activation of a rhodopsin molecule requires 2×10^{-19} J (Yau *et al.*, 1979), 100 times the thermal noise in each dimension, so spontaneous triggering is rare. A photon contains 4×10^{-19} J, easily activating the rhodopsin (Yau *et al.*, 1979). The activated rhodopsin causes hydrolysis of polyphosphatidylinositol in the plasma membrane to yield inositol triphosphate in the cytoplasm. The inositol triphosphate stimulates cGMP release, amplifying the signal. The cGMP binds to ion channels, causing their opening. In a dark-adapted cell, a single photon causes the opening of 1000 channels. The resulting depolarization stimulates neurotransmitter secretion. The neurotransmitter binds to the adjacent neuron and causes it to trigger. The initial stimulus produces a second messenger which starts an amplifying cascade to produce a larger stimulus to the transmission apparatus.

Light also causes the cytoplasmic Ca^{2+} concentration in the rod cells to rise. This increase was previously thought to be part of the perception sequence. However, the release of Ca^{2+} from endoplasmic reticulum by inositol triphosphate (Payne and Fein, 1987) serves to desensitize the cell and is in fact the means of light adaptation; the gain of the cascade is attenuated at least 1000-fold from the dark-adapted state (Yau *et al.*, 1986). The cytoplasmic Ca^{2+} concentration serves as a short-term memory of previous light levels, not as an amplificaton step of signal transduction.

The transduction of sound in the ear is less well established, but is relevant because it occurs through a mechanotransducer, as must gravity perception. A modified form of the sound-detecting cells is used by animals to sense acceleration and maintain balance. In this vestibular system, a calcified sphere called a statolith or an otolith provides the mechanical stimulus in place of sound waves.

In hearing, sound waves reaching the ear enter the cochlea, a remarkable fluid-filled resonance chamber. The cochlea acts as a spectrum analyser by establishing standing waves at different positions for each wavelength. This initial filtering greatly simplifies the amount of information which must be elicited from each sensing cell, since the location of the cell identifies the wavelength, and the intensity of the signal corresponds to the intensity of the sound at that wavelength.

The hair cells of the cochlear epithelium have rod-like microtubule bundles which are displaced by the sound waves in the cochlear fluid. The displacement of the bundles is believed to open stretch-sensitive K^+ channels in the epithelial membrane (Hudspeth, 1985). The influx of K^+ causes cell depolarization, which in turn triggers neurotransmitter release into the synapse with the adjacent auditory neuron.

The cytoplasmic Ca^{2+} concentration increases during stimulation of the hair cells, as it does in retinal rod cells. The sequence of ion movements has been described by Hudspeth (1985). Calcium ions enter from the extracellular pool through voltage-gated Ca^{2+} channels. These channels open during the depolarization caused by K^+ influx. The Ca^{2+} in turn open Ca-gated K^+ channels to the inside of the epithelium where the K^+ concentration is low, allowing the excess K^+ to leave the cell and restore the membrane potential. In effect, the rise in Ca^{2+} serves to attenuate the effect of the stimulus.

What parallels may be drawn between these sensory systems and gravity perception in plants? A plant gravity sensor perceives a mechanical stimulus as do the hair cells in both hearing and balance. The vestibular system uses the inertia of a large mass (a statolith) to detect acceleration. The stimulus is amplified to produce a large signal out of the cell. There is also a means for resetting the conditions in the cell when the stimulus ceases. The perceiving cells transmit a signal to other cells where the final response is elicited. Signalling of this type predominantly uses ion channels to create a rapid response (Methfessel and Sakmann, 1986). A difference between these sensors and plant gravity perception is that nerve cells do not have a plant analogue, so the nature of the transmitted signal will be different. Although the mechanotransducer in *Dionea* trigger-hair bases produces an action potential which may be considered analogous to nervous transmission, the evidence for an action potential being the elicited signal in gravity perception is weak. Also, evidence that cyclic nucleotides have a physiological role in higher plants is not strong, so amplification of the stimulus must occur by some other means. There is one clear commonality between the signals leaving gravity-perceiving cells and those discussed above, and that is polar transmembrane transport.

2. *Calcium Ions in Transduction*

In the sensory transducers just described, Ca^{2+} play an ancillary role. There are also cases where the ion is central in the transduction chain. The intracellular action of Ca^{2+} in regulation is often mediated by Ca-binding proteins, whereby it acts as a second messenger. The best known of these proteins is calmodulin (CaM). Calmodulin, when activated by Ca^{2+}, often regulates enzymes by stimulating their phosphorylation. In this manner, an amplifying cascade is established. Calcium–calmodulin can also directly regulate ion transport proteins (Dieter, 1984). In the sensory transducers discussed above, Ca^{2+} attenuated the signal, but this action was not through CaM. The possible role of Ca–CaM as an amplifier in gravity perception also deserves discussion.

Many enzyme reactions in plants are known to be under Ca–CaM control (Dieter, 1984). Typically, the cytosolic Ca^{2+} concentration is below 1 μM. If

the concentration is elevated beyond this by some stimulus, Ca^{2+} binds CaM. This complex can then bind other enzymes in a regulatory manner.

Perception of light by phytochrome involves Ca–CaM (Roux *et al.*, 1986). One example is the rotation of the chloroplast of *Mougeotia* (Wagner *et al.*, 1984). Red light stimulates phytochrome in or near the plasma membrane, inducing a Ca^{2+} influx from the extracellular medium. This Ca^{2+} binds CaM, in turn stimulating a microfilament-associated protein, perhaps myosin light-chain kinase. The actomyosin then contracts differentially, according to the relative irradiance on phytochrome in the cell, to reorient the chloroplast.

There is good biochemical evidence that Ca–CaM also regulates NAD kinase, NAD–quinate oxidoreductase and ion transport proteins (Dieter, 1984). The first two enzymes catalyse reactions unlikely to be part of gravity perception, though ion transport can be. The broad range of Ca–CaM-regulated reactions in animals suggests that there are many more reactions to be discovered in plants, and that these will have many essential functions.

There is some evidence that CaM may be involved in gravitropism. The evidence is of two kinds: localization of CaM and effects of CaM inhibitors. Immunocytochemical labelling of CaM has identified particularly high concentrations of CaM in gravity-sensing cells of root caps and shoot apices (Roux and Dauwalder, 1985; Lin *et al.*, 1986). In maize roots which require light induction to become positively gravitropic, the CaM activity and graviresponsiveness rise in parallel (Stinemetz and Evans, 1988). Calmodulin inhibitors block gravitropism (Björkman and Leopold, 1987b; Stinemetz and Evans, 1987). Furthermore, CaM inhibitors block the bioelectrical response of maize roots associated with gravity perception (Björkman and Leopold, 1987b).

Signal amplification in perception could occur through Ca–CaM interaction. There is also evidence that gravity perception requires CaM activity. More rigorous experiments are necessary to reveal whether CaM has a direct role in signal amplification during gravity perception.

3. *Phosphoinositides in Transduction*

Signal transduction can also involve phosphoinositides, which were discussed in relation to vision above. There is evidence for the phosphoinositide pathway being present in plants [for review see Poovaiah *et al.* (1987)]. The phosphoinositide pathway is generally initiated by a receptor in the plasma membrane which causes phosphatidylinositol bisphosphate to be cleaved into inositol triphosphate (IP_3) and diacylglycerol by phospholipase C. The IP_3 causes Ca^{2+} release from the endoplasmic reticulum with the consequences described in the discussion of CaM. Diacylglycerol stimulates protein kinase C, which can regulate enzymes by phosphorylation. Protein kinase C has been shown to turn on a class of ion channels which are

completely silent in the unstimulated cell (Strong *et al.*, 1987). Through this pathway there are numerous ways to establish polar transport by a sensing cell, but each requires activation of some receptor in the plasma membrane. There are no experiments to date which have tested the involvement of phosphoinositides in gravity perception.

Signal transduction is accomplished in biological systems in a number of ways. Calcium ions, which have been associated with many aspects of gravitropism, have different roles in different transduction apparatus. In the following sections, I will discuss ways gravity perception may work, using these common transduction pathways as guides.

B. MULTIPLE SYSTEMS

From an engineering standpoint the most efficient way to detect the direction of gravity is to use the displacement by an object which is attracted by gravity—something heavy. Sensors which work in this way are called statoliths. Efficiency and simplicity are two reasons why the statolith theory (Haberlandt, 1900) has been widely accepted for gravity sensing.

There are, however, data which are difficult to reconcile with a statolith theory. In fungi, though gravisensing is poorly studied, it is known that the graviresponse is rapid and that there are no detectable sedimenting bodies (Burnett, 1976). The moss *Physcomitrella* responds to gravity, but no statoliths are apparent (Jenkins *et al.*, 1986). In these, statoliths may have gone unobserved if they are in unexpected places. Bean seedlings provide an example where a simple observation could have failed to find statoliths because the gravisensitive region has been difficult to identify. Etiolated bean shoots perceive gravity using statoliths in the cotyledonary hook (Verbelen *et al.*, 1985), but when de-etiolated they perceive gravity using statoliths along much of the hypocotyl (Heathcote, 1981); in both instances they curve 2–3 cm below the cotyledons. Nevertheless, gravity can apparently be perceived in the absence of statoliths.

Nonstatolith gravity sensing has been proposed in higher plants also. Making amyloplasts too light to sediment by depleting the starch usually eliminates gravisensitivity (Haberlandt, 1902; Iversen, 1969), but there are exceptions. In starch-depleted, excised wheat coleoptiles a graviresponse is observable after 5 h (Pickard and Thimann, 1966). The lighter plastids may have taken longer to have an effect than the normal amyloplasts, which produced a response in excised coleoptiles within 2 h, or a slower alternative mechanism could have been responsible for the graviresponse. A starch-free *Arabidopsis* mutant (Caspar *et al.*, 1985) appears to be a more interesting exception because it responds more quickly. However, changing the intensity of the gravistimulus produces a response fully consistent with the statolith theory (Sack and Kiss, 1988).

In discussions of graviresponsive organs which apparently lack statoliths, the assumption is often made that the same gravisensing system is operating as in organs which do have statoliths (Pickard and Thimann, 1966; Moore and McClelen, 1985). This may not be a safe assumption. The presence of a second mechanism for gravitropism in addition to a statolith mechanism has been proposed (Shen-Miller and Hinchman, 1974); it would not be a novel case of a plant having more than one mechanism for getting an important task done. Gravitropism is essential for many organisms, and it would not be surprising if plants and other organisms are "overbuilt" for responding to gravity, as suggested by George Malacinski (personal communication), because failure to respond to gravity would often have lethal consequences. There may be a sensing mechanism which evolved before plastids which is potentially present in all cells. In organisms with specialized organs and diverse cellular constituents, an efficient statolith-based gravisensing system may operate. While amyloplasts act as statoliths in angiosperms, the green alga *Chara* uses membrane-bound barium sulphate crystals, and both invertebrates and vertebrates use crystalline organic calcium complexes as statoliths in their vestibular (balance) systems. Jellyfish contain calcium sulphate statoliths to detect their orientation relative to the gravitational field. The wide occurrence of statoliths is perhaps an example of convergent evolution. If there are parallel gravity-sensing systems present in an organism and the statolith sensor is disabled, the "primitive" system may take over.

Characterizations of systems with disabled or absent statoliths have concentrated on the rate and extent of curvature, an indicator of how growth is controlled (Moore and McClelen, 1985; Caspar *et al.*, 1985). However, there are no data which indicate whether the gravity-perceiving step has characteristics similar to those in the normal, statolith-containing plants. To learn something about the susception of gravity, the most interesting difference in apparent nonstatolith systems, susception (and perception) must be studied rather than growth. The parameters to measure would be the site of perception, sensitivity to small stimuli, the presentation time and whether the reciprocity rule (Johnsson, 1965) holds.

There are very few data on gravity sensing in organisms which appear never to use statoliths, so there is very little basis for guessing how they might perceive gravity. A critical survey of such mechanisms would be very welcome. Whatever the mechanism is, it must obey the physical laws discussed in this chapter. If an organism truly has no sedimenting intracellular component, the inclination would be to look for relatively large (>20 μm) structures outside the cell, or interactions between cells. If there are indeed parallel gravity-sensing mechanisms, it is important to determine to which mechanism a measured parameter applies. Data gathered from two gravity-sensing systems probably cannot be reconciled as consistent with a single mechanism.

C. STATOLITH SENSORS

1. *Identifying the Statolith*

The most obvious candidates for sensing bodies are those which sediment. Sedimentation of amyloplasts is well documented, and has been the focus of studies on statolith sensors in higher plants. Most cellular components are unable to move freely, being secured by the cytoskeleton. Components which move by cytoplasmic streaming do so by attachment to the cytoskeleton (Wagner, 1979). In concentrating on amyloplasts, have we overlooked other components of the cell which could act as statoliths in addition to, or instead of, amyloplasts?

Intramembrane statolith. If intercellular communication is accomplished by effecting a change in the membrane properties, the plasma membrane may be a good place to look for a sensing body. An intramembrane sensor would be a protein, most probably a transport protein capable of migrating to the bottom of the cell. If a membrane protein could act as a statolith, sedimentation could occur without microscopically detectable changes. Since proteins are more dense than lipids, they would move towards the bottom and not be buoyed to the upper side (Fig. 3a). The sedimentation rate for a protein in the membrane can be calculated from Stokes' Law, using a measured diffusion coefficient for proteins in the membrane (5×10^{-6} m^2 s^{-1}) (Schlessinger *et al.*, 1977) and a typical value for the density of a protein (1.33) and of the plasma membrane (1.03). The time for such a protein to settle half a cell diameter (5 μm) would be 88 years!

Because equilibrium occurs in much less than 88 years, the sedimentation equilibrium is more appropriate to consider than sedimentation kinetics. By this method, we find the difference in concentration of a freely diffusing protein at the bottom of the cell (c) divided by the mean concentration (c_0):

$$c/c_0 = M(1 - \bar{v}\varrho)gh/2RT$$
$$= 6 \times 10^{-8}$$

where M = molecular weight (25 kg mol^{-1}); $\bar{v}$ = specific volume of protein (7×10^{-4} m^3 kg^{-1}); ϱ = density of plasma membrane (1.10×10^3 kg m^{-3}); g = gravity (9.8 m s^{-2}); h = half the height of the cell (5×10^{-6} m); R = gas constant (8 J K^{-1} mol^{-1}); and T = temperature (300 K). Thus, if there were 10^7 molecules of the relevant protein in the membrane there would only be one more molecule in the bottom half of the cell than in the top half at equilibrium. In comparison, a hair cell in the ear contains about 300 transducer channels (Hudspeth, 1985). Clearly gravity will not cause transport proteins to sediment in the plane of the membrane, so sedimentation of proteins will not influence gravitropism.

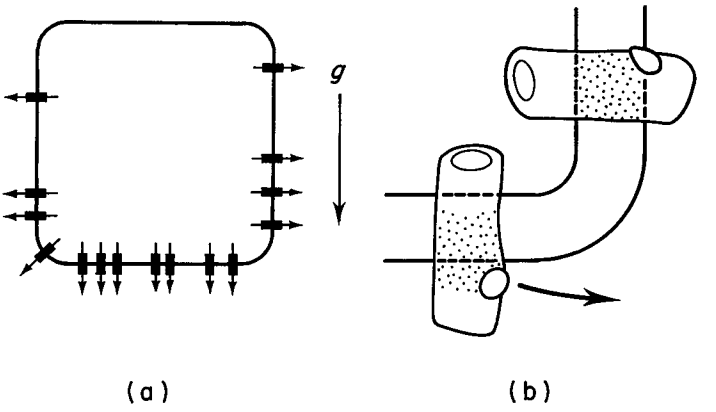

Fig. 3. Hypothetical gravity susception and perception by proteins in the plasma membrane. (a) Dense transport proteins sediment to the lower part of the cell; unequal transport of the substrate creates a gradient. (b) Ion channels are pulled out of the plane of the membrane by gravity, and are active only in this position. This channel would be active only on the lower flank of the cell. Neither mechanism is energetically possible.

In addition to moving in the plane of the plasma membrane, a membrane protein can move across the membrane bilayer. A protein in a horizontal membrane would tend to be pulled out of the plane of the membrane by gravity. If that displacement were great enough to expose a catalytic site, on an ion channel for example, it could act as a gravity sensor (Fig. 3b). As described above, the force of gravity on a single protein is very small, whereas intrinsic membrane proteins are held in position by hydrophobic forces greater than gravity, the thermal motion greatly exceeds the effect of gravity, and the membrane potential also has a stronger influence on the position of the protein than gravity. Gravity therefore cannot be perceived by displacement of proteins across the plane of the membrane.

Membrane components are clearly too small to be effective sedimenting bodies. To find a statolith, it will be necessary to look at larger components, inside the cell.

Intracellular statoliths. To consider which intracellular components could act as statoliths, we will further consider the Stokes equation. The settling rate depends on the difference between the density of the particle and the density of the medium, the particle's volume, and the effective viscosity of the medium. The density of amyloplasts is approximately 1.5×10^3 kg m^{-3}; that of proteins, 1.3×10^3 kg m^{-3}; of mitochondria, 1.2×10^3 kg m^{-3}; and of Golgi bodies, 1.1×10^3 kg m^{-3} (Audus, 1962). The cytoplasm is non-Newtonian, so the viscous drag is difficult to calculate. The effective viscosity and density of the cytoplasm would, however, act similarly on all organelles.

The total work potentially done by a sedimenting particle is the force o gravity times half the cell diameter (for a 90° rotation). Because th cytoplasm must be displaced, the effective sedimenting mass is not densit times volume, but density difference times volume.

For any amyloplast,

$$\begin{aligned}\text{force} &= \text{density difference} \times \text{volume} \times \text{gravity},\\ &= [(1.50 \times 10^3\ \text{kg m}^{-3}) - (1.03 \times 10^3\ \text{kg m}^{-3})]\\ &\quad \times (1.4 \times 10^{-17}\ \text{m}^3) \times (9.8\ \text{m s}^{-2})\\ &= 4.92 \times 10^{-14}\ \text{N}\end{aligned}$$

Potential energy = force × distance, so where distance $= 10^{-5}$ m,

$$\begin{aligned}\text{energy} &= (4.9 \times 10^{-14}\ \text{N}) \times (10^{-5}\ \text{m})\\ &= 4.9 \times 10^{-19}\ \text{J}\end{aligned}$$

The gravitational potential energy in one sedimentable amyloplast is therefore, 250 times the thermal noise ($\frac{1}{2}kT = 2 \times 10^{-21}$ J), about 15 time the stimulus estimated from Fig. 2, and similar to the energy in a photon. A change in angle of 90° was used in this example but plants detect stimuli fa smaller. To account for the observed sensitivity, a 90° rotation shoul generate a response well above the detection limit. The amount of energ involved in amyloplast sedimentation seems reasonable for its proposed rol as a statolith.

Mitochondria are smaller (0.5 μm) and less dense (1.2 g cm^{-3}) tha amyloplasts. They are unlikely to be sensors (Audus, 1962), losing onl 1.1×10^{-21} J ($0.3kT$) of potential energy in a 10 μm fall. Thermal agitatio has a larger effect on mitochondrial motion than does gravity. If a perceivin structure were triggered by such a low energy, it would be triggered b thermal motion far more often than by gravistimulation, even with larg movements. In fact, a mitochondrion would not sediment 10 μm within th presentation time: they have not been observed to sediment at all. Stoke law predicts that other organelles are too small or light to sediment detec ably within the presentation time, and microscopy confirms this (Griffith and Audus, 1964; Edwards and Pickard, 1987).

Sedimenting amyloplasts have been the focus of most studies of th gravity sensor. In addition to the obvious sedimentation of amyloplasts, th great attention given to them is due to the presence of sedimenting amylc plasts at the site of gravisensitivity. Amyloplasts are plastids in which starc storage is the overriding function. Starch storage serves to maintain a energy source without the osmotic consequences of accumulating sma molecules such as hexoses and sucrose. There are many instances c amyloplasts serving only for storage, such as in potato tuber cells and in bea parenchyma, but such amyloplasts maintain a fixed position in the ce (Verbelen *et al.*, 1985). However, in the gravisensing cells of angiosperm

such as the node of grass stems, the root cap, and the gravisensing part of seedling shoots and coleoptiles, there are amyloplasts which sediment in response to gravity. If a maize root is decapped, the quiescent centre will rapidly produce amyloplasts, but gravisensitivity returns only after a change in the cytoplasm which allows them to sediment (Hillman and Wilkins, 1982). The amyloplasts in the gravisensing region are unlike amyloplasts elsewhere in that they are larger, multigranular, will not produce chlorophyll, and have more RNA (Gaynor and Galston, 1983). All gravisensing regions in higher plants normally have sedimenting amyloplasts, and amyloplasts sediment only in gravisensing cells.

Rhizoids of the green alga *Chara* contain membrane-bound crystals of barium sulphate whose sedimentation produces curved growth (Sievers and Schroter, 1971; Schroder, 1904). These statoliths are able to move in a restricted zone in the growing tip of each rhizoid. When the rhizoid is turned from a vertical position, the statoliths sediment rapidly, and collect at the side wall.

The requirements of a statolith are that it be massive enough for displacement to be detected against thermal noise and that it sediments rapidly. The barium sulphate-filled vesicles of the green alga *Chara* meet these requirements, and there may be other kinds in plants. However, in higher plants, the amyloplast is by far the best and apparently only reasonable candidate for a statolith.

2. *Statolith Action*

When susception occurs by sedimentation of a large organelle, what part of sedimentation is actually perceived? There are several aspects of sedimentation which could be detected. These are the position (x), displacement (dx), velocity (dx/dt) and acceleration (dx/dt^2). The statolith balance systems in animals are optimized to detect acceleration. Plants, being stationary, do not need a bioaccelerometer to correct their travel as animals do. The other three aspects of sedimentation can be considered, however.

In this section, how each of these aspects of statolith sedimentation might be the one which is perceived will be discussed. The concept of a pressure sensor is included with displacement, because a pressure transducer works by measuring the displacement of something with specified elastic behaviour.

3. *Statolith Motion*

If statolith velocity is the parameter which is detected, perception would be due to an effect which depends on conditions varying with time. The statolith could affect the structural or the electrical conditions in the cell.

Cytoskeletal shear. The change in gravitational potential energy when an amyloplast sediments was calculated above. How much of the potential

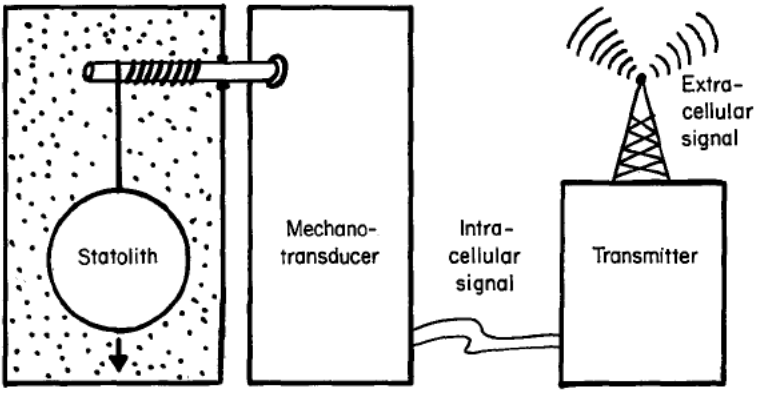

Fig. 4. If a statolith is doing work on a transducer, it will sediment more slowly tha predicted by the viscosity of the medium. The transducer could then modulate the cell communication to other cells.

energy is available to stimulate the transducer? Some portion of the potenti energy is dissipated as heat by the viscous drag of the cytoplasm, leaving th rest available to do work to trigger perception. An analogy is illustrated i Fig. 4. A ball falls through a viscous medium, and a string attached to it turn a shaft on a generator—the analogue of the transducer. The gravitation potential energy is converted to work which is converted to electrical energ in this example. The electrical energy then modulates the signal from th transmitter in proportion to the sedimentation rate. Knowing the mass ar volume of the ball and the viscosity of the medium, one can calculate ho fast the ball would be expected to fall if it were not attached to a transduce If it is attached to the transducer, it will descend more slowly.

Can this analogy be used to determine whether sedimenting amyloplas may be doing work as they sediment? The observed rate of sedimentatic of an amyloplast, 5–20 μm min^{-1} (Sack *et al.*, 1985a), reflects an appare cytoplasmic viscosity of 350–1400 cp (water is 1 cp at 20°C) if the cell considered analogous to a falling-ball viscometer. This viscosity is simil to that of glycerol. Is it appropriate for cytoplasm, or would an unr strained particle fall faster?

The rheological properties of the cytoplasm are believed to be dete mined largely by the cytoskeleton (MacLean-Fletcher and Pollard, 198(Studies of the rheology of actin and microtubule solutions reveal a ve peculiar behaviour. The shear force is essentially constant at all she velocities; i.e. the apparent viscosity decreases in inverse proportion to t amount of stress (Buxbaum *et al.*, 1987). An object moving rapidly throu such a medium will tend to cause its viscosity to decrease, a phenomen called shear thinning. For a fluid with dynamic viscosity characteristics li

those of actin solutions, the behaviour of a falling ball viscometer is difficult to predict, but changes in viscosity would tend to be exaggerated (Rockwell *et al.*, 1984). Therefore, even if the rheological nature of the cytoplasm were known, the expected sedimentation kinetics of amyloplasts could not be calculated accurately. Nevertheless, qualitative assessments can be made.

Experiments on isolated cytosol with a falling-ball viscometer reveal an apparent viscosity of about 1 cp for the sol state, and >1000 cp for the gel state (MacLean-Fletcher and Pollard, 1980). Since the viscometer used a metal ball of different size and density from an amyloplast, the shear forces are different and therefore the viscosity values are not quantitatively comparable. Nevertheless, these measurements indicate that amyloplasts *in vivo* sediment more slowly than isolated amyloplasts would in a sol state cytosol *in vitro*. The possibility remains that amyloplasts are restrained, and may thereby modulate the perceiving mechanism.

An interesting observation is that the amyloplasts slow down as they fall. In corn root statocytes, the initial rate is 19 μm min^{-1}, but after moving about 2 μm, they have slowed to 4 μm min^{-1} (Sack *et al.*, 1985a). Shear thinning of the cytoplasm by Brownian motion of the amyloplast may cause the cytoplasm to have a lower effective viscosity in the region just around the amyloplast. The amyloplast would sediment faster in this thinned region, and slow down when it leaves this region (Fig. 5a). This may in part be the explanation for the observed decrease in the sedimentation rate during the early part of an amyloplast's fall. A region of thinned cytoplasm around the amyloplasts could indicate the gravity vector if it perturbed cell metabolism, which is dependent on the cytoskeletal structure. If this is the case, statolith motion in response to gravistimulation is not detected. Rather, Brownian motion would be used to create a signal which indicates the statolith position.

Alternatively, the obscured slowing could be explained if amyloplasts, like chloroplasts (Witztum and Parthasarathy, 1985), are directly attached to the cytoskeletal matrix. During the initial part of the fall, the amyloplast would not be restrained, but after a short distance, the cytoskeletal elements would become taut and slow the descent of the amyloplast (Fig. 5b). Through this restraint, the motion of the amyloplasts could modulate a signal through localized perturbations as discussed above.

Still another way to account for the slowing of the amyloplasts is compression of the cytoskeletal matrix as the amyloplasts settle on it (Fig. 5c). In *Chara* statoliths are held in position away from the tip of the rhizoid by a cytoskeletal matrix. If the rhizoid is treated with colcemid, the statoliths descend to the extreme apex (Friedrich and Hertel, 1973). If such a compression is involved in perception, displacement rather than motion is detected; this mechanism is discussed later.

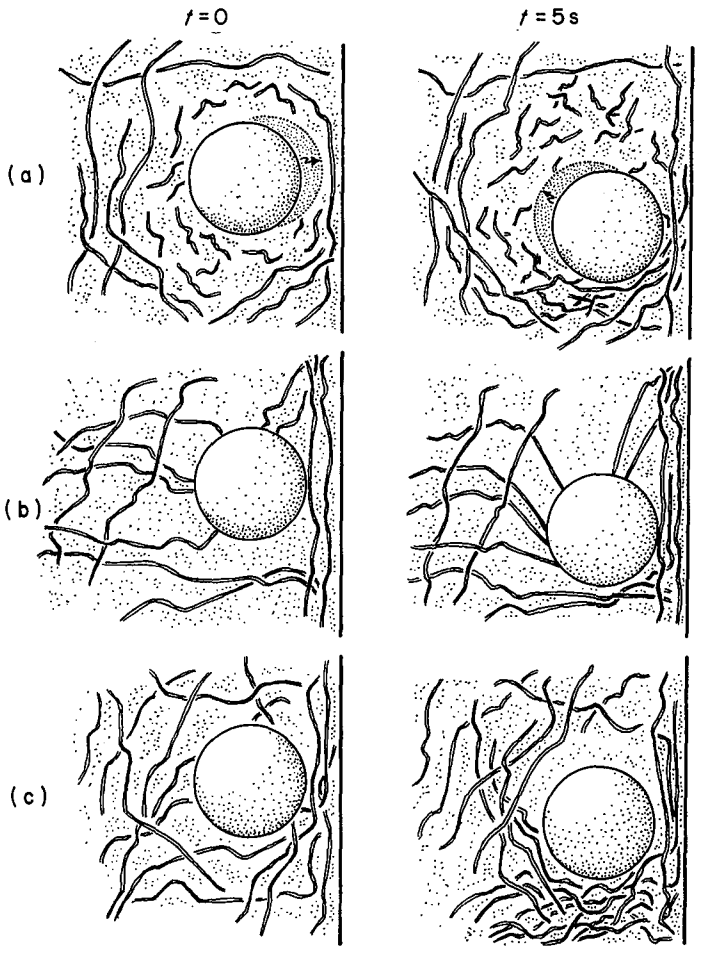

Fig. 5. Models which could explain the slowing of sedimenting amyloplasts. The le column is immediately after gravistimulation, the right column is about five seconds late (a) Shear caused by Brownian motion of the amyloplasts will reduce the effective viscosity microfilaments in the cytoplasm. They will therefore sediment more rapidly through this lay than through the remaining matrix. (b) Hypothesized cytoskeletal connections to amyloplas will become taut after some displacement. (c) Cytoskeletal elements may be compressed belo the sedimenting amyloplast, and increase the effective viscosity.

It is difficult to make more than general suggestions about the significanc of amyloplast sedimentation kinetics based on available data. These model could be tested by measuring the sedimentation kinetics when the statocyte are reinverted. A comparison of these kinetics with sedimentation kinetic of isolated amyloplasts through appropriate actin suspensions *in vitro* woul make it easier to assess the likelihood of connections between amyloplas and the cytoskeleton in detecting amyloplast sedimentation.

Electrical field. An amyloplast carries a charge (Sack and Leopold, 1982), so one can envisage its motion being detected from the changing electrical field. An electrical generator works through electrical and magnetic fields moving past a coil. Bandurski *et al.* (1985) proposed that the amyloplasts deform the electrical field around plasmadesmatal openings and thereby open these intercellular connections, if they are voltage-sensitive. The distortion of the field around the amyloplast would only extend as far as the Debye layer—about 2 nm (Starzak, 1984); even with electron microscopy, this distance is indistinguishable from actual contact, which does not occur (Perbal, 1978; Sack and Leopold, 1985). Also, the potential energy in the charge separation caused by the negatively charged amyloplast moving in the cytoplasm is much less than the potential energy due to gravity. This would be inefficient conversion of the potential energy to work in an instance where almost all the energy is required for sensing to occur within the presentation time.

If none of the potential energy of the amyloplast is converted to work as it sediments, then the sensing must occur when the amyloplast contacts the bottom flank of the cell. The energy available to do work on a sensor by this scheme is the kinetic energy in the moving amyloplast. That energy is also easily calculated using some of the values cited above: kinetic energy depends on the mass and the velocity.

If

$$\text{mass} = 2 \times 10^{-14}\ \text{kg}$$

and

$$\begin{aligned}\text{velocity} &= 20\ \mu\text{m min}^{-1} \\ &= 3.3 \times 10^{-7}\ \text{m s}^{-1}\end{aligned}$$

then

$$\begin{aligned}\text{kinetic energy} &= 1/2 \times \text{mass} \times (\text{velocity})^2 \\ &= (0.5) \times (2 \times 10^{-14}\ \text{kg}) \times (3.3 \times 10^{-7}\ \text{m s}^{-1}) \\ &= 1.1 \times 10^{-27}\ \text{J}\end{aligned}$$

The kinetic energy in a moving amyloplast is four million times less than the thermal noise. This comparison shows that amyloplasts sediment so slowly that there is simply not enough kinetic energy for the motion of an amyloplast to be detected.

Although physiological models to detect statolith motion can be described, energetic evaluations rule them out. Perception based on statolith motion would also fail to explain the continued differential growth after sedimentation is complete until the tissue is again in its preferred orientation. Perception must come about through detection of some parameter of sedimentation other than motion of statoliths.

4. Statolith displacement

An obvious event in gravity-sensing cells is the movement of statoliths fro one position in the cell towards another when the tissue is displaced. Th indicator of orientation in the gravitational field could be the change statolith position. Specifically, the distance statoliths move from the normal (e.g. tissue vertical) position, or the amount some structure displaced by statoliths, could be the graded stimulus which elicits a response

A statolith exerts a force on any structure with which it comes into contac The pressure is detected by displacement of an elastic structure whic responds in proportion to the amount of displacement. A pressure sensor therefore a special case of displacement perception.

The effect of thermal noise on a receptor can be reduced if the recept averages the input over a period of time. If statolith sedimentation do work on a receptor by displacing it, the displacement required for triggerir should be high enough that the triggering by gravity occurs much mo frequently than triggering by thermal agitation of the statolith. This can b accomplished by averaging the signal over time. Although the probability a large thermal displacement increases as the observation period increase the effect of gravity increases more during statolith sedimentation. This ca

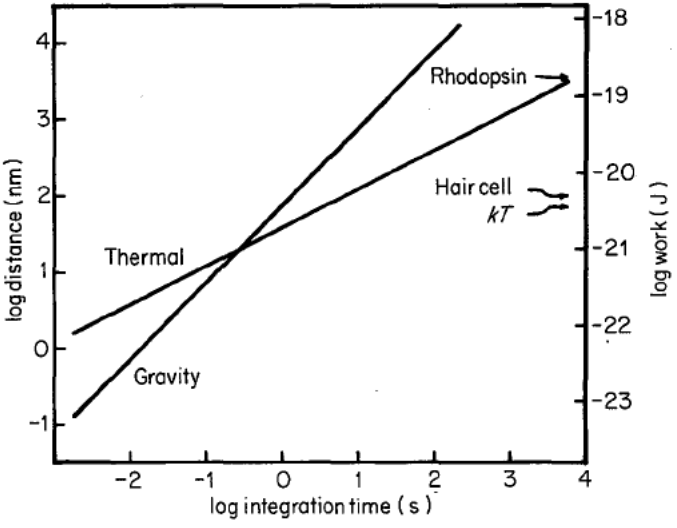

Fig. 6. The effect of integration time on the ability to distinguish the effect of gravity or statolith from the effect of thermal noise. The left axis is statolith motion due to each force. T right axis is the amount of work done by the corresponding displacement of an amylopla Indicated along the right axis is the work required for activation of sensors on vision [rhodops (Yau *et al.*, 1979)] and hearing [hair cell (Corey and Hudspeth, 1983)] as well as thermal noi ($\frac{1}{2}kT$). Values used in this graph were estimated from data in Sack *et al.* (1985a): η = 300 cp 0.3 Pa, r = 1 μm, sedimentation rate = 4 μm min^{-1}. The uncertainty of each line is abc three-fold due to imprecision in the estimation of one of the parameters and assumptions abc the behaviour of the cytoplasm.

be calculated from Einstein's equation of Brownian motion (Einstein, 1907):

$$\sqrt{\overline{\lambda x^2}} = \sqrt{\frac{\tau kT}{\eta r}}$$

where $\sqrt{\overline{\lambda x^2}}$ is the net distance moved, τ is the integration time, η is the viscosity of the medium and r the radius of the particle. The relative effects of sedimentation due to gravity and random thermal motion can be seen in Fig. 6.

An integration of several seconds is needed for sedimentation to be the dominant source of the signal, and for work done by sedimentation to be equivalent to that which triggers other sensitive sensors. Evidence of such integration is that gravistimulation need not be imposed continuously. If short intermittent stimulations are frequent enough, they have the same effect as the same amount of stimulation given continuously (Pickard, 1973b). One-second stimulations every five seconds are summed; half-second stimulations must be repeated every second to be summed. Significantly, the smaller stimuli are "remembered" for a shorter time. The perception mechanism probably averages stimuli over a time period of one or a few seconds, and the minimum stimulus involves a displacement of at least 100 nm.

Cytoskeleton stretching. The lack of contact between amyloplasts and the plasma membrane (Witztum and Parthasarathy, 1985; Heathcote, 1981) suggests that an indirect interaction causes a signal to be passed out of the cell. This indirect interaction could be through cytoskeletal members attached to amyloplasts transmitting, by tension, energy to receptors in a membrane. In the extreme case, where the amyloplasts are completely restrained, they would do no work at all. Amyloplasts are often restrained to this extent in cells which do not function as gravity sensors. The cytoskeleton is certainly a promising agent for transmitting the stimulus, but not by immobilizing the amyloplasts, although they would be partially restrained. This concept has been raised by Larsen (1969) who proposed that the amyloplasts function as pendula attached to the distal part of the statocyte by the cytoskeleton. Stimulus transmission by the cytoskeleton has also been proposed by Shen-Miller and Hinchman (1974) and by Friedrich and Hertel (1973).

If the cytoskeleton is attached to a stretch-sensitive Ca^{2+} channel in the amyloplast envelope, displacement of amyloplasts could cause tension in the stationary cytoskeleton. There is a precedent for microfilament–plastid interaction (Witztum and Pathasarathy, 1985). The tension could cause a conformational change in the channel to which the cytoskeleton is attached, analogous to the way the membrane potential causes a conformational change in voltage-gated channels, by exerting a force on dipoles in the protein. This conformational change decreases the amount of energy needed

to open the channel (Honig *et al.*, 1986). Amyloplasts contain large amount of Ca^{2+} (Chandra *et al.*, 1982). More frequent opening of a Ca^{2+} channel in the amyloplast membrane would cause a locally elevated Ca^{2+} concentration. This region of higher Ca^{2+} would be a directional signal when amyloplasts are only in the lower part of the cell. A Ca^{2+} channel in the amyloplast membrane, attached to microfilaments anchored at the end of the cell where the amyloplasts are in vertical tissues, would cause a rise in Ca^{2+} in the lower part of the cell. A localized release of Ca^{2+} would result in an elevated Ca^{2+} concentration only in a restricted region of the cytoplasm (Keith *et al.* 1985; Brownlee and Wood, 1986; Weir *et al.*, 1987). Should such channel be at the other end of the cytoskeletal connection, in the plasma membrane, sedimenting amyloplasts would not create a directional signal. In that case the local rise in intracellular Ca^{2+} would occur in the same location in the cell regardless of the direction of stimulation.

A locally higher Ca^{2+} concentration around displaced amyloplasts could stimulate ion transport across the plasma membrane in that region of the cell. Elevated cytoplasmic Ca^{2+} may stimulate ion transport protein directly or through CaM (Dieter, 1984). The directional ion transport is the type of signal which the perceiving cell would be expected to elicit.

If sedimenting amyloplasts do work on an ion channel via microfilament or microtubules, they can elicit a physiological asymmetry by stimulating channels in only one part of the cell. The operation of this type of microfilament–membrane channel interaction has been found (Horwitz *et al.* 1986) and is likely to be common (Geiger, 1985). Lawton *et al.* (1986) noted disruption of microtubules around amyloplasts at the onset of sedimentation. The interaction of the cytoskeleton with channels is a promising candidate for perceiving mechanical stimuli.

Displacement of endoplasmic reticulum. Volkmann and Sievers (1979) have proposed that perception is by the interaction of amyloplasts with the endoplasmic reticulum. In that case, amyloplasts would begin to act only after reaching the endoplasmic reticulum at the lower surface of the cell. The presentation time could then be interpreted as including the time required for the amyloplasts to reach a position where they had an effect. Amyloplasts sediment rapidly, as fast as 40 μm min^{-1} in *Taraxacum* stalks (Clifford and Barclay, 1980). Sack *et al.* (1985a,b) measured both the presentation time and the sedimentation of amyloplasts in two different kinds of statocytes—the root cap and the coleoptile of *Zea mays*. In both cases, the amyloplasts sedimented rapidly enough that the first ones had reached the new lower flank of the cell within the presentation time. Sedimentation of amyloplasts thus occurs in an appropriate time period for perception to occur through interaction with the endoplasmic reticulum.

The motion of amyloplasts at the new lower flank of the cell also bear

on this possibility. Heathcote (1981) observed that amyloplasts in *Phaseolus* hypocotyls slow down when they are about 1 μm from the lower wall of the cell. The slowing may be caused by amyloplasts deforming the endoplasmic reticulum which underlies the plasma membrane. Heathcote makes the comment that during sedimentation, the amyloplasts "appeared to be slowed by invisible cytoplasmic structures". Observations of live tissue, like these and also those of Sack (Sack *et al.*, 1985a,b; Sack and Leopold, 1985), are very helpful when considering the interactions of statoliths with the perception mechanism.

Volkmann's observation that graviresponse is proportional to pressure prompted him to propose that the endoplasmic reticulum senses pressure directly (Volkmann, 1974). If the endoplasmic reticulum is deformed elastically, Hooke's law holds that displacement will be proportional to pressure. Hence Volkmann's data support displacement detection equally well.

Sievers *et al.* (1984) propose that the signal which is elicited when amyloplasts settle on the endoplasmic reticulum is intracellular Ca^{2+} released from the endoplasmic reticulum, a storage site for Ca^{2+} in the cell. In their model, statocytes depolarize the cell as a result of Ca^{2+} release when amyloplasts deform the distal beds of endoplasmic reticulum. The proposed involvement of a specific endoplasmic reticulum structure is supported by the observation that a pea mutant in which the endoplasmic reticulum is uniformly distributed is not graviresponsive (Olsen and Iversen, 1980). The endoplasmic reticulum is arranged so that maximum amyloplast contact is with beds on the outer side of the cell (Juniper and French, 1970), explaining the depolarization only of cells on the lower side of the tissue (Behrens *et al.*, 1982). The product of this Ca^{2+}-induced depolarization of the lower cells is an electrical asymmetry across the root cap.

The initial perceiving step remains difficult to explain, namely how amyloplasts cause Ca^{2+} release from the endoplasmic reticulum. Amyloplasts deform the membrane extensively, bringing the endoplasmic reticulum cisternae into contact (Volkmann and Sievers, 1979). Perhaps this intermembrane interaction can induce Ca^{2+} release. The question of how amyloplast action elicits a molecular response is unanswered for this specific model, but the model provides a good tool to answer this question.

5. Statolith Position

Finally, the position of a statolith as the detected aspect of sedimentation will be considered. The argument that statolith position can be detected seems to go against the preceding discussion of thermal noise and of work done during sedimentation, but those restrictions still apply though in a somewhat different way. To detect the position of a statolith there would be a sensitive area on the lower surface of the cell which recognizes the statoliths on some basis other than mass. The statolith would still need to be

massive enough to sediment to the bottom surface without being extensively agitated by Brownian motion. Also, a high-affinity recognition site could use electrical or chemical potential energy rather than gravitational for the recognition (Volkmann, 1974), but the gravitational force would have to be large enough to break the attraction when the tissue is reoriented.

The specific recognition site would be near the outer flank of the cell. Although there is no contact between the amyloplast and the plasma membrane (Perbal, 1978; Heathcote, 1981), there are several structures just inside the plasma membrane with which the amyloplast may interact: one or more layers of endoplasmic reticulum (Juniper and French, 1970; Volkmann, 1974; Sievers and Hensel, 1982) held in place by microfilaments (Hensel, 1984, 1985, 1986b); highly stable cortical microtubules (Kakimoto and Shibaoka, 1986); a meshwork of microfilaments (Parthasarathy, 1985) not involved with cytoplasmic streaming (Derksen *et al.*, 1986) desmotubules which extend into the cytoplasm. There are many structures of importance with which statoliths may interact, but it is unclear which are involved in gravity perception.

Electrostatic attraction. If electrical potential energy is used to trigger perception, it could be through electrostatic attraction between the charged amyloplast membrane (Sack and Leopold, 1982) and charged sites on the sensitive surface. The electrical field created by the surface charge of a membrane decays rapidly away from the membrane surface, being negligible more than 1 or 2 nm from the surface (Starzak, 1984). An electrostatic interaction is not a long-distance one on a cellular scale, requiring an approach microscopically indistinguishable from contact.

Without postulating characteristics of the binding site, it is impossible to calculate whether the energetics of electrostatic binding are reasonable, and there is not enough information for productive speculation about the properties of such a binding site. One generalization which can be applied is that an electrostatic interaction must be strong enough to elicit a reaction but weak enough to allow the gravitational force on the statolith to move it away if the tissue is reoriented.

There is evidence for electrostatic effects of gravistimulation. In maize roots, the surface charge of the plasma membrane changes (Pilet, 1985). This change may affect the transport properties of the charged membrane through screening of substrates and through electrostatic effects on transport proteins within the membrane (Møller and Lundborg, 1985). On the other hand, the plasma membrane surface charge could be altered by changes in the extracellular Ca^{2+} activity (Møller and Lundborg, 1985), which also occurs on gravistimulation (Lee *et al.*, 1983).

Ligand binding. The location of statoliths in a sensing cell may also be detected by releasing chemical potential energy if exothermic binding occurs. The energy to trigger a physiological change would be released by a

reaction such as ligand binding. As a simplistic example, if a ligand on the amyloplast envelope binds to a receptor on the endoplasmic reticulum, an associated ion channel could be caused to open. Cytological evidence suggests that amyloplasts approach the endoplasmic reticulum more closely than they do the plasma membrane (Perbal, 1978). Ligand-activated channels are a common type (Hille, 1984); though ligands are usually small molecules which diffuse quickly in the cytoplasm, none have been found attached to a large organelle. One difficulty in using ligand binding as a perception mechanism in gravity sensing, as with electrostatic binding, is that the binding energy would make it hard for the amyloplasts to come loose again. If the binding is exothermic and the contribution of gravity is energetically negligible, the force of gravity on the amyloplast will be insufficient to release it from the binding site.

A direct role of statolith position in altering growth to produce curvature has been proposed in *Chara* rhizoids (Sievers and Schroter, 1971). An important distinction must be made between this alga and angiosperms, however. Sensing and response occur in the same cell, with the statoliths sedimenting to the exact position in the cell where active growth is occurring. Thus no transmission step is necessary, and perception is presumably simpler. Sievers and Schroter (1971) suggested that the role of the statoliths is to prevent Golgi vesicles from fusing with the plasma membrane and thereby preventing wall growth and membrane expansion in that region of the cell. This contention is strengthened by the observation that growth at the cell apex of vertical roots stops if the statoliths are caused to settle there due to reduced turgor (Sievers and Schroter, 1971), or by treatment with the anti-microtubule agent colcemid (Friedrich and Hertel, 1973). This very straightforward action of statolith position works well in a tip-growing cell, but is difficult to apply to multicellular responses. A statolith acting in this manner must be large in order to remain at the lower flank of the cell despite thermal agitation. Specifically, as described in Section II, it must take about 3×10^{-20} J to move the statolith away in order for thermal displacement to be insignificant, yet for a change in the gravity vector to move the statolith.

Specific chemical or electrostatic interactions are intriguing possibilities. A combination of these attractions with displacement due to gravity would form the basis of such an interaction. There are very few data which are useful in evaluating amyloplast binding, although those which suggest that amyloplast position is important support the possibility indirectly. Based on microscopic evidence, any such direct interaction would most likely be with endoplasmic reticulum or cytoskeletal elements, rather than the plasma membrane or its components.

The identity of the relevant statolith action can thus be limited somewhat. The kinetic energy of statolith motion is too small to be perceived. Statolith position appears to act directly in the case of *Chara* rhizoids. In multicellular tissues, displacement is much more likely. The total displacement of a

statolith on gravistimulation may not be relevant; rather, it may be th displacement of some transducer, occurring only during a part of sedimenta tion. The relevant physical displacement may appear as if statolith positio were detected, as in the model in *Lepidium* root caps involving endoplasmi reticulum beds. The larger response to a sliding action across a cell flank tha to just sedimentation to it (Iversen and Larsen, 1971) implies that a signal i elicited by deformation of a structure on the lower cell flank. Just as there ar several types of biological statoliths, there may be more than one way t perceive their action. For multicellular plants, gravity perception b statoliths is likely to be through the work done by statolith displacement.

D. NONSTATOLITH PERCEPTION

The preceding discussion of statolith action involves fairly straightforwar principles and can refer to a large body of published work because th statolith theory has dominated research in gravitropism. Gravity sensin without statoliths would necessarily be more subtle. There is nevertheles strong evidence that it does occur and the question of how must b addressed. The ability of organisms to detect subtle signals easily exceed our ability to explain it. A remarkable example is the marine mollus *Tritionia* which, without any ferromagnetic particles, detects not only th earth's magnetic field but also the phase of the moon, from the bottom o Puget Sound (Lohmann and Willows, 1987)!

1. Pressure Differential

As an alternative to a sedimenting body, Pickard and Thimann (1966) hav proposed that the weight of a cell's protoplasm stimulates the sensor b exerting pressure on the side of the cell towards gravity. The rationale fo this hypothesis is that the protoplasm is more massive than any substituen of the cell and therefore can exert more force on a sensor. A discussion o this mechanism may be found in Audus (1979). The resultant change i pressure across the cell plasma membrane and wall, higher on the lower side and lower on the upper side, would be instantaneous. This pressure differ ence can be calculated; it is the density of the cytoplasm times gravity time the diameter of the cell. Thus, for a cell 10 μm in diameter, the pressure wil be 0.1 Pa higher at the bottom than at the top. For a large stimulation (90° rotation), the pressure change at the new lower side would be 5×10^{-2} Pa. Plants respond to stimuli that are at least 100 times smaller. The pressure change would have to be detected against the background turgor pressure, which is typically $5\text{–}15 \times 10^5$ Pa. To complicate matters further, the turgo pressure is not static, but is constantly changing with the evaporative demand on the plant and, to a smaller extent, with fluctuations in solute exchange in and out of the cell. In pea stems held in a uniform, humid

environment, the turgor pressure varied over a range of 5×10^4 Pa in the period of 1 min (Cosgrove and Steudle, 1981; Cosgrove and Cleland, 1983). To detect a change in pressure due to gravity against a static background pressure at least ten million times larger, and a rapid fluctuation in that pressure one million times larger, would require an amplifier which would selectively amplify the signal to overcome the noise with only a few seconds of sampling time. Such an amplifier would be unprecedented in both biology and engineering.

2. Membrane Tension

The differential volume change resulting from changes in the osmotic potential inside or outside the cell result in changes in the plasma membrane tension (tangential vector). Because the elastic modulus of the membrane is high, the tension is much more sensitive than the turgor to changes in volume. For reasons very similar to those responsible for the difference in protoplasmic pressure, gravity would cause a difference in membrane tension between the upper and lower surface of the cell. Is there a way this difference in tension could have metabolic consequences leading to cell polarization?

Guharay and Sachs (1984) have discovered a tension-sensitive ion channel which would respond to the tension changes resulting from a change in volume. It represents an attractive candidate for the elusive turgor-sensing mechanism. A direct effect of pressure has been proposed as a turgor pressure sensor (Coster and Zimmermann, 1976), which might be relevant to gravity sensing, but the direct effect of pressure on membrane permeability is relevant only at pressures of 10^8 to 10^9 Pa (Aldridge and Bruner, 1985). A change in turgor always involves a change in cell surface area because cell walls are elastic. This surface area change causes large changes in the membrane tension (Wolfe and Steponkus, 1981) which, through a tension-sensitive channel, may be the basis for turgor sensing.

Although the cell surface area would not change with reorientation in a gravitational field, there would be a differential membrane tension between the top and bottom of the cell. This tension differential has been proposed as a gravity sensor (Edwards and Pickard, 1987). Could the energy from the difference in tension be sufficient to overcome the activation energy for changing the state of the channel? The tension-sensitive ion channel in patch-clamped tobacco protoplast membranes (Falke *et al.*, 1986) opens at a pressure difference of 2500 Pa. If the radius of the patch pipette is 0.5 μm and there is one channel per patch, then the tension for opening and activation energy can be calculated:

$$\begin{aligned}\text{Tension} &= 1/2(\text{pressure difference}) \times (\text{radius of curvature}) \\ &= 1/2(2.5 \times 10^3 \text{ Pa})(5 \times 10^{-7} \text{ m}) \\ &= 625 \ \mu\text{N m}^{-1}\end{aligned}$$

$$\begin{aligned}\text{Energy} &= (\text{tension}) \times (\text{area of patch}) \\ &= (6.25 \times 10^{-4}\,\text{N m}^{-1}) \times 1/2(4\pi)(5 \times 10^{-7}\,\text{m})^2 \\ &= 10^{-15}\,\text{J}\end{aligned}$$

The activation energy for channel opening is about 10^{-15} J per channel occurring at a tension of 625 μN m^{-1}. For comparison, the resting tension of a plant cell membrane is about 100 μN m^{-1} and the critical tension for lysis is 4000 μN m^{-1} (Wolfe and Steponkus, 1981). The difference in tension due to gravity, based on a turgor difference of 0.05 Pa and a cell radius of 5 μm, is about 0.25 μN m^{-1}. Even if the energy in the differential tension caused by gravity across a whole cell's membrane ($<3 \times 10^{-16}$ J) could be focused on a single channel, it would not overcome the activation energy for opening (10^{-15} J).

This particular channel would not be useful as a gravity sensor, but could a more sensitive channel work, e.g. a channel with an activation energy around 3×10^{-20} J? A channel with this higher sensitivity, at a density of 100 per cell, would be activated at 0.002 μN m^{-1}. Such a channel would be constantly activated by the resting tension of the membrane (>100 μN m^{-1}). A tension-sensing channel is only effective if it is sensitive to changes relative to the resting tension, so the minimum tension for opening must be greater than the resting tension. A very specialized channel could conceivably open over a narrow range, e.g. between 100 and 100.25 μN m^{-1}. However, normal fluctuations in the resting tension are much larger than this (Wolfe and Steponkus, 1981), so the channel would not really be capable of detecting changes due to gravity. A channel which opens at a tension meaningfully higher than the resting tension would require more energy for activation than that which gravity provides to the cell. There is no design by which a change in the membrane tension due to gravity can be detected by a tension-sensitive channel.

There are other difficulties with perceiving gravity using membrane tension. The cell tension is constantly being adjusted through addition and removal of membrane material, so that small differences such as that due to gravity would be difficult to distinguish. Further, while it is clear how the absolute tension of a structural part of the cell can transmit energy to a channel, there is no obvious way to interpret a differential tension in a similar way.

In walled cells, the radius of curvature of the membrane is much smaller than in protoplasts because it is appressed to the microfibril meshwork of the wall. Therefore the tension in the membranes of turgid walled cells is much higher than described above. If tension sensors were connected to the wall (Edwards and Pickard, 1987), the tension would be higher than that calculated for the membrane, but the differential tension would be the same. Since wall tension is directly related to turgor, the arguments regarding turgor in the previous section apply to this model as well. Detection of

changes in wall tension with a stretch-sensitive channel is even more difficult than detection of changes in membrane tension.

The mechanotransducer of *Dionea* appears to detect pressure on sensing cells from the trigger hair, but the pressure change is much larger, being produced by deflection of a multicellular trigger hair which acts as a lever. These pressure changes would be of the same order as detectable turgor changes, so a turgor sensor based on membrane or wall tension is a physically reasonable mechanotransducer in *Dionea*.

Moore *et al.* (1984) imply that there is a nonstatolith sensor in decapped corn roots which keeps them from regenerating caps in space. There are two explanations which have not been tested. First, without a $1 \times g$ control in space, the effects of radiation cannot be determined. That these effects are likely to be important is illustrated by the effect of gravity on space-grown lettuce hypocotyls. Relative to ground controls, the growth rate of seedlings in space was inhibited by 30% whether at microgravity or at $1 \times g$ (Merkis *et al.*, 1985). Thus some condition in space other than gravity depressed the growth rate. Differences between earth- and space-grown material are not necessarily due to gravity. Cell division—which is initiated in quiescent zone cells during cap regeneration—appears to be particularly sensitive to radiation in space vehicles (Kostina *et al.*, 1984). Second, the mitotic apparatus may be large enough to act as a statolith. This does not appear to have been tested. In light of the many reasons why root cap regeneration might fail in spaceflight, it appears unnecessary in this case to invoke a nonstatolith gravity sensor.

The basic assumption in the above two schemes involving the mass of the protoplasm is that the mass of the whole cell is greater than any individual part, and can therefore be the source of more energy than any individual part. These schemes then depend on focusing that energy to do work on one or a few sites. Both schemes are unreasonable because, based on the above calculations, energy depends both on mass and displacement. While the whole cell is a larger mass, it is not displaced relative to itself; no work is done, so there is no energy to focus on a receptor.

3. *Multicellular Sensors*

A nonstatolith gravity sensor would not have to be intracellular. The entire organ could be the sensor of gravity, with the weight of the organ compressing the lower tissue and stretching the upper tissue. This effect would be apparent immediately upon gravistimulation, and would provide a large force. Sliwinski and Salisbury (1985) investigated this possibility by restraining the shoot so that it was curved upward, thus producing compression on the upper surface whereas the stem would normally be compressed on the lower side. Despite this reversal of tension and compression, the shoot curved normally. Thus this mechanism has been rejected experimentally for a higher plant shoot.

A gravisensing root is normally supported by the soil matrix, and doe not experience stresses similar to those of a horizontal plant part in air Pressure of the tissue against the matrix below it is not the signal to roots since roots curve similarly in soil and in humid air. Also, by bending th terminal 1–2 mm of root tips (the cap but not the elongating zone) in glas tubes, the two parts could be gravistimulated separately. Subsequen curved growth in these roots occurred if the root cap was horizontal an the elongating zone vertical, but not if the cap was vertical and th elongating zone horizontal (Pfeffer, 1894). This experiment elegantl excludes stresses on the whole tissue in roots.

When discussing a multicellular gravity sensor it is important to distin guish the gravitropic reaction from reaction wood formation which i indirectly a response to gravity. Reaction wood formation appears to b associated with responses like thigmotropism and seismonasty, wher mechanical stress causes altered growth mediated by ethylene formation.

It appears that an extracellular gravity sensor based on tissue stresses doe not exist in higher plants. However, there appear to be no experiments i fungi or mosses, where there are no obvious statoliths, which test such a hypothesis. In large plants, the force of gravity produces a thigmic stress such as that produced by wind or contact. The response to thigmic stresse occurs by a different mechanism. The physical constraints on how a multi cellular gravity sensor would operate are great, and make it difficult t design a hypothetical model of such a system.

V. INTEGRATION AND CONCLUSION

There has been much speculation about what the gravity sensor may be; speculation will continue until a definitive causal role for sedimenting statoliths (amyloplasts in higher plants) has been proven or another scheme is found which requires no statoliths. Much of this speculation has ignored fundamental rules of the physical world: (1) gravity can only interact with a mass; (2) a mass must move to do work on a sensor; (3) the energy of the motion must be detectable in the presence of thermal noise. In a system as small as a cell the energy of thermal noise dominates; in a system as large as a human being, thermal noise is practically unnoticeable. Therefore, our conception of what can be detected inside a single cell could easily be wrong. A mass which senses gravity must be large on a cellular scale, and it must move observable distances under the influence of gravity, if it is to affect the physiology of a gravisensing cell.

There are two lower limits to activation of perception—thermal noise and energy of activation. Susception must produce a signal large enough to trigger perception. To prevent frequent spontaneous triggering, the necessary stimulus must be several times greater than thermal energy. Specifically,

to meet these requirements gravity perception will have an activation energy of about 4×10^{-21} J.

There is compelling evidence for the existence of both statolith and nonstatolith gravity perception. It is not known whether these can be simultaneously operating in one graviresponsive issue. Models of perception without statoliths have yet to satisfy the basic physical requirements of signal transduction. The scant information available to date is not sufficient to create relevant models. The interaction of a statolith with the mechanism which perceives sedimentation must use the potential energy which is released during sedimentation. The kinetic energy of a moving amyloplast is too small to be detected; the work done by statolith displacement is the most reasonable interaction. This work is most likely done by deforming a cellular structure during some part of sedimentation, resulting in a physiological response.

The integration of the gravitational stimulus through discontinuous stimulation experiments (Pickard, 1973b) can be correlated with intracellular events to test hypotheses about the initial physiological steps of gravity perception. One such hypothesis is that membrane depolarization is the integrator as in retinal rod cells. The cytoplasmic Ca^{2+} concentration can also act as an integrator if statolith sedimentation produces a graded Ca^{2+} release. The mechanism of stimulus integration appears to be an accessible step of perception to study.

For progress in the study of gravity perception, it is essential to be able to study individual steps in gravitropism. Although it is unlikely that steps of gravity sensing can be isolated and studied *in vitro*, it must be possible to detect the function of individual steps. Of particular interest would be to correlate a signal intensity with details of statolith behaviour. It would then be possible to test hypotheses which predict that specific interactions between statoliths and other cellular components give rise to perception.

To test models of gravity perception invoking elevated cytoplasmic Ca^{2+} concentrations as an intracellular signal, it is imperative to determine whether the Ca^{2+} concentration really changes within the cell during gravistimulation, and, if so, where in the cell. The role of Ca^{2+}, phosphoinositides and other second messengers in other examples of signal transduction has been assessed by microinjecting the substance into the sensing cell, and measuring the elicited signal. This technique could easily be applied to gravity perception if the elicited signal could be measured. Curvature is a poor parameter to use because it is a highly variable, delayed response which requires optimum function of many unknown intermediate steps. The various models which include release of Ca^{2+} into the cytoplasm invoke different sources for that Ca^{2+}: extracellular, endoplasmic reticulum and plastid. If cytoplasmic Ca^{2+} does increase with gravistimulation, it would be very useful to find the immediate source of that Ca^{2+}.

Finally, a robust study of sensitivity and noise discrimination has yielded

a groundwork for evaluating susception in hearing. An equivalent study of graviperception could restrict the possible mechanisms to a few, and would put to rest much of the controversy about the statolith hypothesis. In this paper, the author has been able to impose only very wide limits using available data.

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