Chapter 4

Gravity Dependent Processes and Intracellular Motion

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ABSTRACT

Most organelles large enough to sediment or to undergo isothermal settling within eukaryotic cells are held in position by one or more components of the cytoskeleton. The interior of eukaryotic cells is considered to be very crowded, and the evaluation of natural-convective processes is very difficult. In a most simple view, the cell may be considered as consisting of four immiscible phases among which solutes are exchanged causing steep concentration gradients and thermodynamic conditions far from equilibrium. Extracellular gravity-related forces may include natural convection due to solute gradients external to single cells or the work performed by swimming, ciliated, or elongating cells.

The purpose of this essay is to introduce the biophysicists' view, which is to be elaborated upon by other authors in this volume. If we think of the cell as a suspension of Stokes' particles in a Newtonian fluid, which it is not (see below), then we might treat the chromosome, as an example of an organelle, as a Stokes' particle with finite dimensions and a drag force, a buoyant force, and a gravitational force, and balance these forces and calculate its velocity. From this exercise we would come to the conclusion that a metaphase chromosome travels at about $2 \times 10^{-7}$ centimeters per second by simply falling within the cytoplasm. This is roughly the same velocity, within a factor of two, as the observed velocity of migration during mitosis (Figure 1, Todd, 1977, 1989). We know that there is movement of the chromosome in the cell during mitosis and that it is driven by the metabolic energy of the cell and guided by microtubules, but a closer look at the chromosome inside the cell tells us that there are numerous fibers in the cell, and chromatin is sticking out in numerous directions. We do not even yet know with certainty whether or not chromatin strands actually connect one chromosome to another. There is evidence (Lewin, 1981) in some plants that it does, and there is little or no evidence to the contrary.

In the growing root tip of a plant, the cells are not only elongating vertically but they are also dividing vertically. Thus questions are raised about the effect of gravity on orientation. Gravity could directly orient the metaphase (dividing) cell, or its action could be indirect, so that the cell is responding to the action of gravity on larger objects, which in turn signals the mitotic cell that it is to divide vertically or horizontally and signals the interphase cell whether it is to elongate or not.

The cytoskeleton can immobilize organelles inside the cell, but this was discovered quite a number of years after plant physiologists reported that the amyloplasts (starch granules) that occur in the statocytes of the growing root tip sediment downward. The amyloplasts are some half a dozen $\mu$m in diameter; the cells are large and the amyloplast density is 1.3-1.5 in normal plants. So which effects dominate—those due to gravity or those due to the cytoskeleton? This depends on the cell. Evidence over the past few years is forcing us to argue that the cell capable of gravity perception, at least in the plant root tip, may be one in which the cytoskeleton is adjusted so that gravity-dependent internal motion of organelles is possible. When $g$ (or inertial acceleration) is used as an experimental variable, the rate of movement and the position of the amyloplasts is modified (Evans et al., 1986). When the roots are held pointing down, the amyloplasts are found near the bottom of the cell, and when the root is held sideways the amyloplasts are also at the bottom, where “bottom” is defined by the gravity vector. This change occurs in minutes.

The gravity signal is still transduced when starch is not synthesized and is absent from the amyloplast. In certain mutant cells that produce no starch the amyloplast density is substantially lower, and there is argument that either these objects can still function (at least partially) in their graviception capacity when starch is not being synthesized (Kiss et al., 1989) or, the alternative hypothesis, that they have no role in graviception.

Concerning Stokes' sedimentation in this context, Dr. Brown (Chapter 1, this volume) alluded to intracellular settling and the papers of Pollard (1965, 1971) and others, including Tobias et al. (1973), which address the role of Brownian movement. Brownian movement in a sense counterbalances sedimentation in the case of very small particles, less than about 0.5 $\mu$m in diameter and below a density difference of about 0.03 g/cm$^3$. The result of this counterbalance is called isothermal settling and is similar to events in the atmosphere. Over very long ranges the high-molecular weight molecules are packed more densely at the bottom of the atmosphere. This type of a gradient is set up.
through the same small volume and similarly for 0.4 \mu m particles, etc., as shown in Figure 2. The same calculations can be repeated using various density differences.

If the physical properties of certain organelles are surveyed, all could sediment within the cell on the basis of their diameters if they were considered to be suspended in an unconfined Newtonian fluid (Table II). But all organelles are attached to something and they tend not to move around inside the cell. It has been seen that if a cell is touched on one end with a microelectrode, for example, the other end sometimes moves. This is true at least in mammalian cells, in which the cytoplasm holds its particulate contents tightly in place through the cytoskeleton. This role of the cytoskeleton is a major issue, as are the dynamics of the cytoskeleton in intracellular graviception. The cytoskeleton is actually part of the metabolic machinery of the cell. The actinomyosin fibers of the cytoskeleton have ATPase functions, and the ATPase functions can translate chemical energy into mechanical energy.

Figure 1. Treatment of the eukaryotic chromosome as a sedimentating object (Todd, 1977). Calculations are given in Table I.

Table I. Hydrodynamic Values for a Metaphase Chromosome (see Figure 1) Used for Application to the Sedimentation of Isolated Chromosomes (Todd 1977, 1989)

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>2\pi r^2 l = 25 \times 10^{-12} \text{ cm}^3</td>
</tr>
<tr>
<td>g</td>
<td>980 \text{ cm/sec}^2</td>
</tr>
<tr>
<td>\rho - \rho_0</td>
<td>1.35 - 1.04 = 0.31 \text{ g/cm}^3</td>
</tr>
<tr>
<td>3\sqrt{3V/4\pi}</td>
<td>2.1 \times 10^{-4} \text{ cm}</td>
</tr>
<tr>
<td>\eta</td>
<td>5 \pm 2 \text{ dyn-sec/cm}^2</td>
</tr>
<tr>
<td>v</td>
<td>2 \times 10^{-7} \text{ cm/sec}</td>
</tr>
</tbody>
</table>

on the basis of the Boltzmann distribution in which the concentration of particles c(h) at height h relative to the concentration of particles at the bottom c(0) goes as

\[
c(h)/c(0) = \exp[-V (\rho - \rho_0) gh/kT]
\]

where the exponential term is simply the buoyant settling energy of the particle divided by kT, the thermal, or Boltzmann, energy (Pollard, 1965; Tobias et al., 1973). If we consider a collection of 0.2 \mu m particles, suspended in a 20 \mu m vessel, possibly a cell, then the particles will be distributed isothermally and adiabatically in the vertical direction along a slope corresponding to that of the lower curve in Figure 2, with many more at the bottom than at the top. If we reduce gravity by a factor of 100 to 0.01 g, those same 0.2 \mu m particles will be almost uniformly distributed.

A number of cells do work against gravity. When a nerve cell puts out a process (neurite formation) in vitro (Figure 3), it traverses many microscopic fields in order to make a connection to a distant cell millimeters away. That cell may be doing work against gravity. Kessler (Chapter 10, this volume) discusses cells that swim, especially large ones that do work against gravity. From the animal physiologist’s standpoint, there is a counterpart; the cell that sits still and moves things with its cilia may also be doing work against gravity.

One of the roles of the cytoskeleton is to hold organelles in place. Well over a decade ago David Prescott performed a series of experiments with cells treated with cytochalasin B. This compound causes the disaggregation of cytoskeletal microfilaments that are composed principally of actin. It was possible to subject any kind of a cell, usually mammal-
lian cells attached to some surface at one side, to an inertial acceleration away from the attachment plane and simply pull the nucleus away from the cell and leave an anuclear "cytoplasm."

It may be of interest to explore the cell as a four-phase system. The separation of two immiscible aqueous polymer solutions into two phases is probably a naive and simplistic analog of how the cell separates into several phases, but we must consider that the cell has "non-aqueous phases" which are the membranes, and an aqueous phase which is the cytoplasm (or cytosol). In addition, there are certain places in the Golgi cisternae and usually the exterior of the entire cell, whether it be an animal cell or a plant protoplast, which are covered with a high concentration of polysaccharides. This high concentration of polysaccharides has very different physical properties from the aqueous medium in general. The solubilities of other solutes are different, the viscosity is different, and presumably things like heat capacity and conductivity are also different from those found in simple aqueous solutions.

There are parts of the cell that can probably be legitimately called solids, and this notion is articulated in an interesting theoretical, actually speculative, discussion by Fulton (1982) in which she summarized work in which cells

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### Table II. Physical Properties of Organelles Used to Calculate Stokes' Sedimentation Velocities $\nu$; $x$ and $t$ are sedimentation distances and corresponding times, respectively.

<table>
<thead>
<tr>
<th>ORGANELLE</th>
<th>VOL ($\mu$m$^3$)</th>
<th>$\rho$ (g/cm$^3$)</th>
<th>$\rho-\rho_0$ (g/cm$^3$)</th>
<th>$\nu$ (cm/sec)</th>
<th>$t$ (sec)</th>
<th>$x$ ($\mu$m)</th>
<th>FEATURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MITOCHONDRION</td>
<td>2-100</td>
<td>1.1</td>
<td>.01-.02</td>
<td>1x10$^{-8}$</td>
<td>10$^3$</td>
<td>0.1</td>
<td>Convoluted structure</td>
</tr>
<tr>
<td>NUCLEOLUS</td>
<td>10-20</td>
<td>1.4</td>
<td>0.3</td>
<td>2x10$^{-7}$</td>
<td>10$^4$</td>
<td>20</td>
<td>Suspended by chromatin</td>
</tr>
<tr>
<td>CHROMOSOME</td>
<td>5-50</td>
<td>1.4</td>
<td>0.3</td>
<td>2x10$^{-7}$</td>
<td>10$^3$</td>
<td>2</td>
<td>Suspended by microtubules</td>
</tr>
<tr>
<td>AMYLOPLAST</td>
<td>100</td>
<td>1.5</td>
<td>0.4</td>
<td>1x10$^{-6}$</td>
<td>&lt;10$^3$</td>
<td>10</td>
<td>Sedimenting particle</td>
</tr>
<tr>
<td>OTOLITH</td>
<td>1000</td>
<td>2.0</td>
<td>0.8</td>
<td>&gt;1x10$^{-5}$</td>
<td>1</td>
<td>0.1</td>
<td>Known g-sensor</td>
</tr>
<tr>
<td>DICTYOSOME</td>
<td>100</td>
<td>1.2</td>
<td>0.15</td>
<td>&gt;3x10$^{-7}$</td>
<td>10$^3$</td>
<td>2</td>
<td>Internal membrane</td>
</tr>
</tbody>
</table>

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from a variety of sources had been dehydrated and rehydrated. She then asked the question — what metabolic functions can occur under various conditions of hydration?

Another interesting question is whether or not convection can occur intracellularly (Kessler, 1978; Pollard, 1965; Todd, 1989). Albrecht-Buehler (Chapter 3, this volume) discusses this subject, but we tend to ignore convection at small dimensions. However, convection is considered a significant issue in the low-gravity experiments that have dealt with the growth of protein crystals (DeLucas et al., 1986). In some senses these processes may not differ from the self-assembly processes that occur inside the cell (Ataka and Asai, 1988). There are at least two events that could lead to convection. One is the removal of solute from the immediate vicinity of the growing crystal surface, resulting in a reduction in the density of the fluid surrounding it. The other is the heat of crystallization being released to the fluid around it and raising its temperature. This leads us to ask additional questions — can the cell be considered as an isothermal system, and is the cell a system functioning near equilibrium (during development we think of the cell as being far from equilibrium)?

The application of simple (and complicated) physical principles to the action of gravitational acceleration inside the cell depends heavily on our understanding of the physical properties of the cell interior. While substantial progress has been made in the last decade toward understanding cytoskeletal structure and bioenergetics, the dynamics of intracellular and intercellular forces requires considerable further study.

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REFERENCES


