Graviperception and Graviresponse at the Cellular Level

Richard Bräucker, Augusto Cogoli and Ruth Hemmersbach

The evolution of life on Earth occurred under the persistent influence of gravity. Even protists (unicellular organisms such as flagellates and ciliates) had to find and to stay in environments whose chemical and physical parameters fit their needs. Consequently, already unicellular organisms developed organelles for active (oriented) movement (cilia, flagella) and sensors for diverse stimuli. Among environmental parameters, gravitational acceleration is a most reliable reference for orientation, because it is virtually constant in its magnitude and direction. Consequently, graviorientation can be already found on very early, unicellular, stages of development [1-3]. As protists are heavier than water, they had to develop mechanisms to compensate sedimentation. Without graviorientation, a population of Paramecium, for instance, would sink to the ground of a 1m depth pond within 3½ hours.

The mode of gravibehavior in protozoa seems to depend on the preferred living conditions. Paramecium which feeds on aerobe bacteria, shows a negative gravitaxis, i.e. the cell population swims mainly upwards, against the gravity vector, thus reaching oxygen saturated layers. In contrast, Loxodes which prefers low oxygen concentrations, shows a positive or a bimodal gravitaxis [4]: alternately moving upwards or downwards increases the chance of finding optimal conditions of light and O\textsubscript{2} concentrations which change with the depth of the water column in an aquatic ecosystem (Fig. 18.1).

In addition to gravitaxis (the oriented movement with respect to gravity), some species show a gravity related active regulation of swimming rate, called gravikinesis. This results in an increased swimming rate during upward swimming, and a decreased swimming rate during downward swimming.

18.1 Protists

18.1.1 Perception of Varied Acceleration

Changing the magnitude of a stimulus is a common method to study its influence on a living system. Experiments under microgravity conditions as in space flights, sounding rockets and drop towers are necessary to confirm that gravitational acceleration is the reason of the phenomena examined [5-7]. Furthermore, it was demonstrated that a step transition from 1 \times g to microgravity in a drop tower led to an unexpected slow tran-
sient loss of graviresponses, which gives some hint on the nature of the gravireceptor in ciliates.

Using a centrifuge in microgravity environment gives the chance of investigating the behavior of cells under hypogravity conditions and during application of a distinct acceleration force for threshold studies. In search for the threshold of graviperception, graviresponses were documented in ciliates and flagellates at 0.1 to 0.3 $\times g$. Long-term cultivation for up to 14 days in $\mu g$ did not show dramatic changes in morphology of the protists although the proliferation rate increased [8]. Behavioral changes due to the lack in gravity (Fig. 18.1B) were restored after return to 1 $\times g$ conditions [9].

Hypergravity conditions which can be achieved on earth with comparatively low expense, showed no saturation of gravireactions in ciliates up to 5 $\times g$ conditions [10-11].

From these experiments it may be speculated that perceiving of and reacting to gravitational forces is not the limiting factor for survival of ciliates on other planets (Table 18.1.), whereas most remaining physical or chemical parameters (temperature, pressure, atmosphere, radiation) will probably be past endurance of protists.

**Table 18.1.** Equatorial escape velocity and equalorial surface gravity of several celestial bodies

<table>
<thead>
<tr>
<th>Celestial body</th>
<th>Moon</th>
<th>Mars</th>
<th>Venus</th>
<th>Earth</th>
<th>Jupiter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escape velocity [km s$^{-1}$]</td>
<td>2.38</td>
<td>5.02</td>
<td>10.36</td>
<td>11.18</td>
<td>59.56</td>
</tr>
<tr>
<td>Surface gravity [m s$^{-2}$]</td>
<td>1.62</td>
<td>3.72</td>
<td>8.87</td>
<td>9.78</td>
<td>22.88</td>
</tr>
<tr>
<td>Equatorial surface gravity [g]</td>
<td>0.166</td>
<td>0.380</td>
<td>0.879</td>
<td>1</td>
<td>2.339</td>
</tr>
</tbody>
</table>

Understanding the mechanisms of graviperception in protists would possibly help to
also answer questions on the role of gravity in smaller cell systems such as mammalian cells.

18.1.2 Models of Graviperception

Since the detailed description of the negative gravitaxis (orientation against the direction of gravity) of the ciliate *Paramecium* at the end of the 19th century [12], several hypotheses tried to explain this behavior, either assuming physical or physiological mechanisms (for review see [1-3]).

While "static" hypotheses try to explain graviorientation as a result of unequal mass distributions inside the cytoplasm (buoy-like mechanism) [12-14], "hydrodynamic" hypotheses suppose that the shape of the cell causes a gravitaxis via different sinking speeds of the anterior and posterior hemisphere of the cell [15].

Since ciliates are actively swimming organisms, some authors presumed the difference between a cellular center of gravity and a center of propulsion to be the reason of graviorientation [16].

Major arguments against a pure physical mechanism of gravitaxis in protists are the facts that immobilized cells sediment in variable orientation of their longitudinal axis [17] and that the direction of gravitaxis switches under special circumstances without changes of the cell form [18, 19].

Alternative models of graviperception assume that the cell is capable of active perceiving the direction of the gravity vector and orients itself actively.

It has been proposed that a *Paramecium* either senses the hydrostatic pressure difference between its upper and lower hemisphere (hydrostatic hypothesis [20]), or that the cell swims into the direction of increased resistance (i.e. upwards) by measuring its energy consumption [21]. Both latter assumptions presume storage and temporal evaluation of measured values, which is unlikely in a unicellular organism.

Alternatively, other authors [22-25] suggested a statocyst-like mechanism, assuming that heavy compartments of the cell act as statolith inducing a selective stimulation of the locomotion apparatus. This mechanism would be similar to graviperception in plants (see Chap. 19, Schnabl). Due to the lack of a morphological distinct statocyst-like organelle in most ciliates, it was proposed that in *Paramecium* the mass of the whole cytoplasm, whose density exceeds the density of the surrounding medium by 4%, causes a mechanical load on the lower cell membrane, thus stimulating gravisensors ("statocyst hypothesis"). Detailed knowledge about the connection of cellular membrane potential and swimming behavior (electromotor coupling, [26]) and the cognition of distributions of mechanically sensitive ion channels [27] allowed proving this hypothesis in behavioral experiments.

In *Paramecium*, the ciliary activity and thus the swimming behavior is under the rigid control of the membrane potential. Hyperpolarization (shift of the negative membrane potential to more negative values) increases the swimming rate, whereas depolarization (shift of the membrane potential to more positive values) leads to decreased swimming rates or even backward swimming. Mechano-sensitive ion channels are located in a characteristic, bipolar distribution in the plasma membrane: mechano-sensitive K-channels mainly posteriorly and mechano-sensitive Ca-channels mainly anteriorly (Fig. 18.2). Stimulation of these channels leads to either depolarization (Ca-
channels) or hyperpolarization (K-channels) of the membrane.

In an upward swimming cell, a gravistimulation of the posterior hemisphere hyperpolarizes the membrane potential, resulting in an increased swimming rate. In a downward swimming cell, stimulation of the anterior cell pole leads to decreased swimming rate. The consequence is gravikinesis which has been found in several ciliate species [1, 28, 6]. From electrophysiological studies, we have first evidences of a gravireceptor potential in *Paramecium* depending on the orientation of the cell with
Break-down of an existing calcium gradient by means of the ionophore calcimycin (A23187), as well as manipulating the membrane potential by means of the lipophilic cation triphenylmethylphosphonium (TPMP\(^+\)) [30] resulted in a loss of gravitaxis in the flagellate *Euglena*. This also confirms that changes in the membrane potential of the cell are involved in graviperception.

Further support of the statocyst hypothesis came from density experiments. Increasing density of the medium (Ficoll or Percoll) impaired gravitaxis of *Euglena* and *Paramecium*. Orientation was completely disturbed at 1.04 to 1.05 g/cm\(^3\), which corresponds to the mean cell density. Higher densities than the density of the cells reversed the direction of movement [31, 32]. These results indicate that intracellular organelles are not involved in graviperception of *Euglena* and *Paramecium*. In contrast, the ciliate *Loxodes* maintained its gravitaxis independently of the density of the surrounding medium, thus demonstrating the existence of an additional intracellular gravireceptor [32-34] (compare Chap. 20, Anken et al). These so-called Müller organelles or Müller vesicles are specialized vacuoles containing a heavy body of BaSO\(_4\) which is fixed to a modified cilium.

Destruction of the Müller organelles by means of a laser beam leads to a loss of the gravitactic orientation in *Loxodes* [32], demonstrating its role in gravity sensing. It can be concluded that different graviperception mechanisms have been evolved in several systems in parallel, but is there a limit concerning cell size for gravisensation?

### 18.1.3 Energy Considerations

Gravikinesis and gravitaxis have been demonstrated in several protists of various sizes. Especially regarding the smaller species the question arises, whether the mechanical load of the cytoplasm is sufficient for signal transduction via mechanosensitive (= gravisensitive?) ion channels in the lower cell membrane. Calculations have given the values listed in Table 18.2.

Compared to the thermal noise level (2·10\(^{-21}\) J) the signal to noise ratio is sufficient for *Bursaria*, *Paramecium* and *Tetrahymena*, but may be critical in *Euglena* and even smaller cell systems such as lymphocytes. These energetic considerations, slow re-

<table>
<thead>
<tr>
<th>Species</th>
<th>Volume [µm(^3)]</th>
<th>Gating energy [J]</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bursaria truncatella</em></td>
<td>3.0×10(^7)</td>
<td>1.2×10(^{-16})</td>
<td>[28]</td>
</tr>
<tr>
<td><em>Paramecium caudatum</em></td>
<td>3.3×10(^5)</td>
<td>4.6×10(^{-19})</td>
<td>[35]</td>
</tr>
<tr>
<td><em>Tetrahymena pyriformis</em></td>
<td>2.2×10(^4)</td>
<td>6.5×10(^{-20})</td>
<td>[36]</td>
</tr>
<tr>
<td><em>Euglena gracilis</em></td>
<td>2.6×10(^3)</td>
<td>4.0×10(^{-21})</td>
<td>[36]</td>
</tr>
</tbody>
</table>

response times (10 s to 60 s [5]), as well as threshold values in the range of 0.1 ×g to 0.3 ×g (determined by means of a centrifuge microscope in space) [37, 38, 9] demand...
the involvement of supporting structures such as the microfilament system. This assumption is supported by results from electric field experiments [39].

18.1.4 Gravisensory Channels

The gravisensory channels in protists have not yet been identified. They may be stretch activated or linear activated channels [40, 31]. The use of channel blockers provided controversial results so far, obviously due to their lack of selectivity. Gadolinium has been used in the study of graviperception, as it blocks the gravitropism in plants [41] and the gravitaxis in *Euglena* [31, 42]. Therefore, it was postulated that stretch-activated channels (SACs) in the cell membrane are stimulated by the sedimenting cell mass. However, similar results could not be achieved in case of *Paramecium*, neither by behavioral nor by electrophysiological studies [43], indicating that gadolinium is not specific for mechanosensitive channels in this species. Nagel and Machemer postulated that the mechanoreceptors in *Paramecium* are not activated by stretch, but by a pressure acting perpendicular to the cell membrane [43] (see also Chap. 20, Anken et al.). Energetical considerations support this hypothesis: The threshold value for the tension of stretch activated channels has been suggested $10^{-4}$ N/m [44] possibly being too large for graviperception in small cells. However, linear activated mechanoreceptors in the hair cells of the inner ear of vertebrates [45] require much lower activation energies, better fitting the data calculated for small ciliates such as *Tetrahymena* (Table 18.2) [36].

Recent studies concentrate on the potential role of second messengers in the gravi-transduction chain [42]. In ciliates, second messengers such as cyclic adenosinemono-phosphate (cAMP) and cyclic guanosinemonophosphate (cGMP) are involved in behavioral responses, though there are still questions concerning the time course of events in the signal transduction chain. Due to current knowledge, cAMP is coupled to hyperpolarization events and cGMP to depolarization events in *Paramecium* [46]. Biochemical assays of cells which had been exposed to varied acceleration levels (by means of centrifuge, sounding rocket flights) are currently under investigation and will help to elucidate the role of second messengers in graviperception.

18.2 Mammalian Cells

18.2.1 Gravity Effects and Consequences

Studies under varied acceleration conditions demonstrated the capability of free living cells to adapt quickly to different environments, but what is the situation in higher specialized cells such as mammalian cells? The answer of this question is not only essential for understanding the complex machinery of cells but also with respect to manned space missions.

In an experiment carried out in 1983 in Spacelab 1 it was discovered that mitogenic activation of T lymphocytes was nearly completely inhibited at µg [47]. A follow-on experiment in Spacelab D-1 revealed that lymphocytes were highly damaged under
microgravity conditions, whereas cells cultured at $1 \times g$ in flight on a $1 \times g$–reference centrifuge undergo mitosis and blastogenesis (Cogoli et al. in [48]). Ultrastructural changes observed by electron microscopy suggested that programmed cell death (apoptosis) is increased in microgravity. This hypothesis was later supported by biochemical and microscopical studies [49, 50].

It was also shown that white blood cells are capable of autonomous movements, of cell-cell contacts and of the formation of aggregates in microgravity [51]. This again was a surprising and unpredictable finding as it was thought that mammalian cells which lack special locomotion organelles, can move only on a substratum and gravity is somehow driving the motion. It was also seen that the cytoskeleton which plays an important role in signal transduction, undergoes structural changes few seconds after exposure to $\mu g$ [52]. A major break-through was achieved in studies on the self-assembly of microtubules at $\mu g$ [53]. Microtubules self organize \textit{in vitro} in a reaction-diffusion process, which appears to be gravity-dependent. In fact, in a 13 minutes flight on a sounding rocket, microtubules did almost show no self-organization in the absence of gravity compared to samples in an in-flight 1g-centrifuge [53]. Such findings are of primary importance because they point to a direct effect of gravity on basic structural elements of the cell.

Signal transduction is an extremely complicated process involving membrane receptors, G-proteins, the cytoskeleton, several protein kinases, transcription factors and oncogenes. Limouse et al. [54] and de Groot et al. [55] were the firsts to investigate intermediate steps of signal transduction in space. They reported that protein kinase C (PKC) is inhibited $\mu g$ [54], and furthermore that the intracellular distribution of this important enzyme is changed in microgravity [56]. In addition, the genetic expression of the early oncogenes \textit{c-fos, c-myc, c-jun} was found to be significantly depressed in $\mu g$. Genetic expression was remarkably changed also in T-lymphocytes exposed to simulated microgravity in the random positioning simulator, also called 3-D clinostat [57]. Finally, using DNA microarrays, it was shown recently that the expression of 1632 genes was changed in human renal cortical cells cultured for 8 d in space [58].

In summary, data from experiments with different mammalian cell systems show that gravity may influence signal transduction pathways by altering the function of the cytoskeleton (probably via protein G), of important enzymes such as PKC, and of the expression of several genes, in particular of oncogenes and cytokines. As the signal transduction paths are essentially similar in all animal cells, it can be assumed that some of the effects seen in lymphocytes may be detected also in other cells. It is important to notice that gravitational effects are seen mainly in cells undergoing differentiation rather than in quiescent or non-differentiating cells. If differentiation is the response to the reception of a specific signal and consists of new expression of specific genes, we can expect that cancer cells which are dividing spontaneously (i.e. without the intervention of specific signal), are less or not at all dependent on gravitational changes.

Several tests were also conducted on lymphocytes from space crewmembers \textit{in vivo}, and in parallel to the investigations \textit{in vitro} (reviewed in [18]). Thereby, it was seen that the conditions of space flight (launch, in flight and landing activities) cause in more than 50% of the subjects significant depression of the cellular immune response,
which is known to be T cell dependent. Such effect is induced by the psychological and physical stress of space flight on the neuroendocrine/neuroimmune system rather than by a direct effect of weightlessness *per se*. Nevertheless, the depression of the immune system may become a serious health issue on prolonged space missions such as a flight to and from Mars.

### 18.3 Conclusions

Studies under varied acceleration conditions demonstrated that free living cells such as protists are able to perceive changes of the acceleration conditions. Recent studies favor the hypothesis that in these systems gravity is perceived either by intracellular receptors (statocyst-like organelles), heavy cell organelles (such as nucleus) and/or by sensing the cell mass by means of ion channels located in the cell membrane.

Mammalian cells in microgravity were profoundly influenced. Alteration in the cellular mechanisms and structures in mammalian cells like signal transduction and the cytoskeleton were detected. It can be speculated that the depression of the immune system may become a serious health issue on flights to and from Mars.

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