

The Effects of Microgravity on Cells

A brief literature review of the effects of gravity and microgravity on a range of cell types.

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Astronauts experience profound physiological changes as they adjust to the microgravity environment in space. We know that all physiological processes in the body are governed by the activity of diverse and specialized cells which comprise the different tissues. Thus, spaceflight exerts its detrimental effects on astronauts via changes in cellular structure and or functions. Numerous studies have shown that all types of cells from many organisms behave differently in space than on Earth. Because there is a great diversity of cell types in nature, the effects of microgravity on those cells are extremely diverse and often complex. To begin understanding those effects we must first understand what gravity physically does to cells **(1, 2)**. In summary gravity causes cells: 1) to be under hydrostatic pressure (compression), 2) to have different cellular organelles (like the nucleus and mitochondria) accelerated at different rates, 3) to experience their own mass as weight, and 4) to be subjected to thermal convection currents in the fluid around them (fluid mixing).

All major physical components of gravity can have important effects on cell function. For instance, compression due to hydrostatic pressure, can affect the internal load-bearing structure of the cell (actin, microfilament, and microtubule cytoskeleton) making it more or less resistant to compression. In microgravity the cytoskeleton would be expected to become less prominent because of less need to support load due to hydrostatic pressure. Flight experiments have shown such cytoskeletal effects **(3, 4, 5, 6)**. Another prominent effect of hydrostatic pressure due to gravity is the adhesive compression of the cell against a rigid substrate (or other cells). Cell adhesion under normal gravity is one of the best understood cell and molecular biology topics. The adhesive molecules (integrins, and extracellular matrix) are exquisitely well known and the signaling responses of the cells (via kinases and transcription factors) are also well documented. In brief cells contain internal cytoskeletal actin stress fibers that end in adhesive molecule (integrin) clusters that then stick to the matrix outside the cell. These structures are called focal adhesions. We know from centrifugation (hypergravity) studies that the greater the gravity, the more focal adhesions are formed **(7)**. In microgravity these structures are likely to be reduced in number **(8)**. These focal adhesions also contain clusters of signaling molecules (kinases) that regulate gene expression programs. For instance 50-g can turn the cellular proliferation gene expression program, making more bone cells **(7)**. On the other hand, microgravity has been shown to reduce proliferation of bone cells, presumably because focal adhesion and other mechanical interactions are reduced in the absence of gravity **(9)**. The cell's own weight (factor 3) is likely to have the same type of effects as hydrostatic pressure. The second physical component of gravity we list, differential acceleration of cellular organelles is best documented by gravitropism studies of plant cells. In plants, starch containing plastids (statoliths), are cellular organelles denser than the rest of the cytoplasm. Because of this greater density at unit gravity, statoliths sediment to the bottom of the cell. The plant cell then detects the position of the statoliths and directs root cellular proliferation (growth) in that direction (gravitropism) **(10, 11)**. In microgravity this process is disrupted, but can be restored by artificial gravity (centrifugation) **(12)**. The molecular mechanism for the signal transduction of the gravity information in plants is still not well understood, even though this is one of the clearer examples of a biological

gravity sensor. In all nucleated (eukariotic) cells the nucleus is also a potential gravity sensor. The tightly packed DNA in the nucleus is about 20% denser than the rest of the cell. In normal 1-g this would tend to make the nucleus sink to the bottom of the cell, however the cytoskeleton actively maintains the nucleus in place. This process transfers a nuclear load to the cytoskeletal fibers adjacent to the nucleus and could be another way of transducing information about the direction and magnitude of the gravity field the cell is subjected to **(1)**. Although this effect is postulated, no molecular mechanism for sensing nuclear positioning is known yet.

The final physical component of gravity relevant to cells we list is thermal convection. At 1-g, heated fluids rise to the top along the gravity vector. Heated fluids are then exchanged by cooler fluids, establishing a convection current that rapidly dissipates heat, renews nutrient supplies, and removes waste materials **(1, 2)**. This factor is most important where no fluid flow (like blood flow) exists to dissipate metabolic products and exchange nutrients around the cell. Without convection, slow diffusion processes are the only means for heat and nutrient exchange. This factor is likely to be most relevant in plants **(13)** and single celled microorganisms as some bacteria that have no motile structures like cilia or flagella. Although blood flow in animal tissues mostly overcomes this effect, this could still be a factor in cells localized where blood flow is minimal.

Having described how we conceive gravity affects cells, we must now examine and summarize what research has been done in this field and what are the current directions investigators are taking. A current search of Medline indexed publications that use cells and microgravity as keywords yields in excess of 1000 publications (1190). However under closer scrutiny most of these papers are ground-based microgravity model studies (randomization of the gravity vector) or hypergravity (centrifugation) studies. Only about a third of these publications actually study cells in microgravity. Many initial studies concentrated on the fine structure (morphology) of cells grown in microgravity and looked at issues like position of organelles, cytoskeletal density and organization, nuclear structure etc **(14, 15, 16, 9)**. Many studies were also performed at the cellular and tissue level focusing on how cells migrate and position themselves during development in the frog **(17)**, how fertilization and development occurs **(18, 19)**, how neurons migrate **(16)** and many others. Some of those studies have discovered only subtle or no effects at the structural level **(18)**, however in other cases the structural effects are substantial. The most prominent microgravity-induced cellular and tissue differences have been identified in muscle tissue cells **(14)**, bone tissue cells **(9)**, and in immune system cells **(20)**. In all three cases, structural abnormalities correlate to observed bone, muscle and immune disorders observed as a result of spaceflight. Another class of cellular studies focuses on metabolic and signaling pathways **(21)**. These biochemical studies of cells have yielded results showing that many important pathways are affected by microgravity. Among these are basic energy metabolic pathways, and proliferative (mitogenic) pathways. As a result in changes of these pathways cellular functions like migration, growth/division, survival are altered by microgravity. More recently studies have started to be conducted at the genomic level **(22, 23)**. In these studies gene arrays are used to determine which genes are expressed at the level of message RNA by cells in microgravity. This message RNA is ultimately translated into proteins that direct every function of the cell from signaling to structure.

Gene array studies of cells grown in microgravity show remarkable effects. There are many-fold increases and decreases of message levels for many (hundreds) genes in microgravity. This means that cells are mounting a massive gene regulatory effort to acclimate to the microgravity environment and maintain homeostasis. Even more recent studies (proposed and undergoing) are focusing on proteins expression (proteomics). Few proteomics results exist yet, however studies of selected protein levels in cells show many are altered in response to microgravity (24), again suggesting rapid cellular adjustment to the space environment. All these studies address the immediate adjustment to a one-factor (gravity) changes, a biological phenomenon termed acclimation. None of the cellular studies so far has focused in-depth on long-term adaptation to the space environment over multiple generation of cell growth and organism reproduction because of the experiment length constraints. Future cell experiments on the ISS will likely focus on this among other questions.

References With Supporting Abstracts - (Relevant text is bold and italicized)

1) ASGSB Bull 1989 Aug;2:95-113

Gravity-dependent phenomena at the scale of the single cell. Todd P. Chemical Engineering Science Division, National Institute of Standards and Technology, Boulder, CO 80303, USA. Progress in gravitational cell biology research will depend on the continuing evaluation of a wide variety of physical phenomena affected by gravity and their roles in extracellular, intercellular, and intracellular processes. This paper examines those *responses of organisms to gravity which depend on functions at the single cell level*. Single cell functions are affected by perturbations in their internal and external environment by a variety of factors, one of which is the effect of gravity. *Physical phenomena that could influence cell function include sedimentation, buoyancy-driven convection, streaming potential, hydrostatic pressure, and interactions among physical transport processes. Thermal motion and fluid viscosity play a significant role in all transport processes at the cellular level. The sedimentation of intracellular organelles tends to be counteracted by the cytoskeleton.* Intracellular convective transport may be possible in large cells. In a microgravity environment extracellular solutes must be transported by diffusion or active circulatory processes in the absence of density gradient-driven convection, and flocculation and coalescence are reduced by the lack of motion of aggregates. PMID: 11540086 [PubMed - indexed for MEDLINE]

2) ASGSB Bull 1991 Jul;4(2):25-34

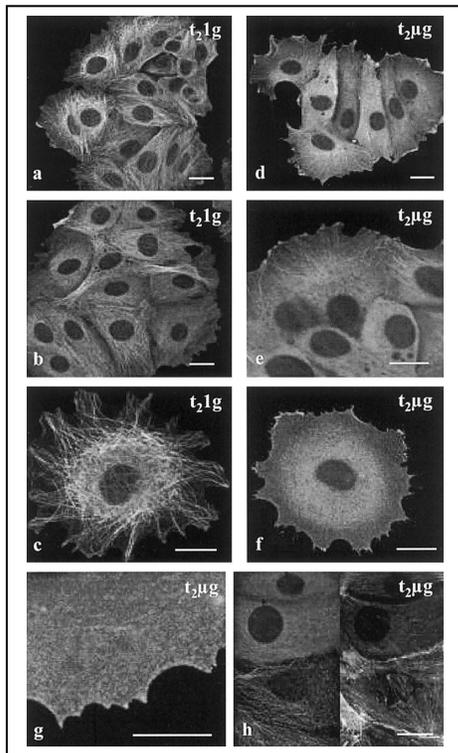
Possible mechanisms of indirect gravity sensing by cells. Albrecht-Buehler G. Department of Cell, Molecular and Structural Biology, Northwestern University Medical School, Chicago, IL 60611.

We have to distinguish between (a) direct gravisensing, in which specialized cells function as parts of a gravisensing organ and (b) indirect gravisensing, in which other cells that have no specialized gravity detectors are nevertheless affected by the inertial acceleration. In both cases, cells may detect (a) the direction of gravity ("up" versus "down"), and /or (b) the amplitude (0 - 1 g) of gravity. This chapter argues that the weight of single normal-sized cells (approximately 10 microns in diameter) is too small compared with other cellular forces to allow them the distinction between up and down. However, the weight of the surrounding medium is much larger. Cells may be able to sense certain environmental changes caused by gravity and thus may sense indirectly at least the amplitude of gravitational forces. *In particular, the fluid environment of the cell can be expected at normal gravity to support microconvective currents that cease to flow at microgravity. Thus, the absence of gravity may be transduced into the accumulation of metabolites and ions from the cells and depletion of fresh nutrients. These changes, in turn, can affect the contacts of cells, their membrane potential, their cytoskeleton, and thus, ultimately, their behavior.* As to ground-based simulations of microgravity, the above considerations suggest that the averaging of the vectorial force of gravity in clinorotation is inadequate for simulation because it may actually

increase rather than suppress convective mixing above the normal levels. PMID: 11537179 [PubMed - indexed for MEDLINE]

3) FASEB J 2001 Apr;15(6):1104-6

The effect of weightlessness on cytoskeleton architecture and proliferation of human breast cancer cell line MCF-7. Vassy J, Portet S, Beil M, Millot G, Fauvel-Lafeve F, Karniguian A, Gasset G, Irinopoulou T, Calvo F, Rigaut JP, Schoevaert D. AIPC Lab., Universite Paris 7, IUH, Hopital Saint Louis, 1 avenue Claude Vellefaux, 75475 Paris cedex 10, France. jvassy@chu-stlouis.fr



In μg , cell spreading was reduced and the number of Ki-67 positive cells (cycling cells) was increased. The experiment was performed in the IBIS instrument (Instrument de Biologie Spatiale) developed by the CNES (French Centre National d'Etudes Spatiales). IBIS contains two sets of cassettes: one was kept in weightlessness (10–5 residual gravity, μg), while the other was kept in a 1g centrifuge (1g in-flight control). Human breast cancer cells MCF-7, flown in space in a photon capsule, were fixed in 1.5% paraformaldehyde and 0.1% glutaraldehyde after 1.5 (t0), 22 (t1), and 48 h (t2) in orbit. Time t0, just before the 1g in-flight centrifuge started, was selected to study the effects of launching stress; for example, vibrations and acceleration.

Cells subjected to weightlessness (abbreviated by μg) were compared with 1g in-flight and ground controls. Double fluorescent labeling was used to detect Ki-67 (nuclear antigen, found in cycling cells) and microfilaments (Texas Red-phalloidin, TR-phalloidin) simultaneously. TR-phalloidin

staining was used to assess cell spreading and to count the total number of cells (Ki-67 positive and negative one) in each image. Ki-67 positive cells were recognizable by their nuclear and nucleolar staining (FITC) in interphase cells (most of them) or by chromosome staining in mitotic cells. Noncycling cells at the time of fixation were Ki-67 negative. At time t2 (48 h after launching), cells were more fully spread in 1g in-flight control than in μg . The fraction of cycling MCF-7 cells was calculated as the ratio of the number of Ki-67 positive cells/total number of cells. When comparing 1g in-flight controls and μg , cycling cells were significantly more numerous in μg than in 1g at time t2 ($P=0.011$) and t1 ($P=0.046$). PMID: 11292682 [PubMed - indexed for MEDLINE].

4) Adv Space Res 2001;28(4):529-35

Microtubule self-organisation depends upon gravity. Tabony J, Pochon N, Papaseit C. Commissariat a l'Energie Atomique, Departement de Biologie Moleculaire et

Structurale, Laboratoire de Resonance Magnetique en Biologie Metabolique, C.E.A., Grenoble, France. jtabony@cea.fr

The molecular processes by which gravity is transduced into biological systems are poorly, if at all, understood. Under equilibrium conditions, chemical and biochemical structures do not depend upon gravity. It has been proposed that biological systems might show a gravity dependence by way of the bifurcation properties of certain types of non-linear chemical reactions that are far-from-equilibrium. We have found that *in-vitro preparations of microtubules, an important element of the cellular cytoskeleton*, show this type of behaviour. *On earth, the solutions show macroscopic self-ordering*, and the morphology of the structures that form depend upon the orientation of the sample with respect to gravity at a critical moment at an early stage in the development of the self-organised state. *An experiment carried out in a sounding rocket, showed that as predicted by theories of this type, no self-organisation occurs when the microtubules are assembled under low gravity conditions*. This is an experimental demonstration of how a very simple biochemical system, containing only two molecules, can be gravity sensitive. At a molecular level this behaviour results from an interaction of gravity with macroscopic concentration and density fluctuations that arise from the processes of microtubule contraction and elongation. ©2001 COSPAR. Published by Elsevier Science Ltd. All rights reserved. PMID: 11799984 [PubMed - indexed for MEDLINE]

5) Acta Astronaut 2001 Aug-Nov;49(3-10):399-418

Spaceflight and clinorotation cause cytoskeleton and mitochondria changes and increases in apoptosis in cultured cells. Schatten H, Lewis ML, Chakrabarti A. Department of Veterinary Pathobiology, University of Missouri-Columbia, Columbia, MO 65211, USA.

The cytoskeleton is a complex network of fibers that is sensitive to environmental factors including microgravity and altered gravitational forces. Cellular functions such as transport of cell organelles depend on cytoskeletal integrity; regulation of cytoskeletal activity plays a role in cell maintenance, cell division, and apoptosis. Here we report cytoskeletal and mitochondria alterations in cultured human lymphocyte (Jurkat) cells after exposure to spaceflight and in insect cells of *Drosophila melanogaster* (Schneider S-1) after exposure to conditions created by clinostat rotation. *Jurkat cells were flown on the space shuttle in Biorack cassettes* while Schneider S-1 cells were exposed to altered gravity forces as produced by clinostat rotation. The effects of both treatments were similar in the different cell types. Fifty percent of cells displayed effects on the microtubule network in both cell lines. Under these experimental conditions mitochondria clustering and morphological alterations of mitochondrial cristae was observed to various degrees after 4 and 48 hours of culture. Jurkat cells underwent cell divisions during exposure to spaceflight but a large number of apoptotic cells was also observed. Similar results were obtained in Schneider S-1 cells cultured under clinostat rotation. *Both cell lines displayed mitochondria abnormalities and mitochondria clustering toward one side of the cells which is interpreted to be the result of microtubule disruption and failure of mitochondria transport along microtubules*. The number of mitochondria was increased in cells exposed to altered gravity while cristae morphology was severely affected indicating altered mitochondria function. These results

show that *spaceflight* as well as altered gravity produced by clinostat rotation *affects microtubule* and mitochondria organization and results in increases in apoptosis. Grant numbers: NAG 10-0224, NAG2-985. c 2001. Elsevier Science Ltd. All rights reserved. PMID: 11669127 [PubMed - indexed for MEDLINE]

6) Biol Sci Space 1994 Jun;8(2):79-93

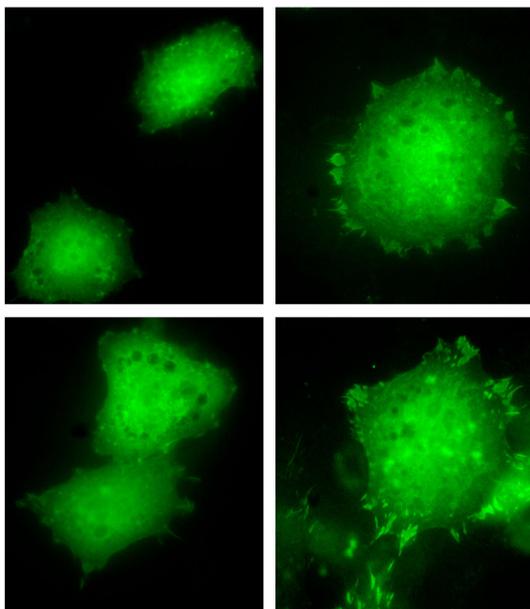
Reduced receptor aggregation and altered cytoskeleton in cultured myocytes after space-flight. Gruener R, Roberts R, Reitstetter R. Department of Physiology, University of Arizona, Tucson 85724, USA.

We carried out parallel experiments first on the slow clinostat and then in space-flight to examine the effects of altered gravity on the aggregation of the nicotinic acetylcholine receptors and the structure of the cytoskeleton in cultured *Xenopus* embryonic muscle cells. By examining the concordance between results from space flight and the clinostat, we tested whether the slow clinostat is a relevant simulation paradigm. *Space-flown cells showed marked changes in the distribution and organization of actin filaments* and had a reduced incidence of acetylcholine receptor aggregates at the site of contact with polystyrene beads. Similar effects were found after clinostat rotation. The sensitivity of synaptic receptor aggregation and cytoskeletal morphology suggests that in the microgravity of space cell behavior may be importantly altered. PMID: 11542735 [PubMed - indexed for MEDLINE]

7) NASA Astrobiology Science Conference 2002, Apr. p32

Integrin-Mediated Cellular Response to Hypergravity. Vercoutere W, Roden C, Searby N, Globus R, Almeida EA. NASA Ames Research Center

Focal Adhesions at 1 (left) and 50-g (right). At 50-g early focal adhesion formation after adhesion (30min) is greatly enhanced relative to 1-g controls.



Microgravity is a serious obstacle to space exploration since prolonged weightlessness results in significant atrophy of musculoskeletal tissues. Cell growth and survival in these tissues are regulated by several environmental signals, including contact with extracellular matrix (ECM), other cells, and soluble growth and survival factors. Kinase signaling pathways involved in survival and proliferation can also be activated by mechanical forces. We hypothesize that gravity is a component of the mechanical environment needed for the efficient transduction of cell growth and survival signals from the ECM. Therefore, reduction of the potential gravitational component of survival

signaling during spaceflight may promote cell cycle arrest and increase programmed cell death (apoptosis) and significantly contribute to the musculoskeletal tissue atrophy observed in response to microgravity. Our previous studies have shown that the ECM component fibronectin strongly supports cell survival in the absence of other soluble growth and survival factors in primary synovial fibroblasts (Ilic et al., 1998, JCB; Almeida et al., 2000, JCB) and that fibronectin is critical for the survival of bone-forming osteoblasts. In fibroblasts this survival signal is transduced by integrins and focal adhesion kinase (FAK), and results in the formation of signaling complexes at focal adhesion sites and a well-organized cytoskeleton. ***In our current studies we show that mechanical stimulation of primary osteoblasts from hypergravity stimulates the activation of FAK and promotes matrix-dependent cell survival.*** Conversely, we observed that the rotating wall vessel microgravity model interrupted matrix-dependent cell survival pathways and increased apoptosis.

8) FASEB J 2001 Sep;15(11):2036-8

Cell cycling determines integrin-mediated adhesion in osteoblastic ROS 17/2.8 cells exposed to space-related conditions. Guignandon A, Lafage-Proust MH, Usson Y, Laroche N, Caillot-Augusseau A, Alexandre C, Vico L. Laboratoire de Biologie et de Biochimie du Tissu Osseux-Equipe Mixte INSERM E9901, Universite Jean Monnet, Saint-Etienne, 15 rue Ambroise Pare, F-42023 Saint-Etienne Cedex 2, Grenoble, France. guignand@univ-st-etienne.fr

Six days of microgravity (Bion10 mission) induced dramatic shape changes in ROS 17/2.8 osteoblasts (7). During the Foton 11 and 12 space flights, we studied the kinetics (0-4 days) of ROS 17/2.8 morphology and adhesion, the relationships between adhesion and cell cycle progression after 4 days in space, and osteoblastic growth and activity after 6 days in space. Quantitative analysis of high-resolution adhesion [focal adhesion area imaged by total interference reflection fluorescent microscopy (TIRFM)] and integrin-dependent adhesion (imaged on confocal microscope by vinculin and phosphotyrosine staining) as well as cell cycle phase classification [Ki-67 staining, S-G2, mitotic cells and G1 (postmitotic cells)] were performed using programs validated in parabolic flight and clinostat. ***We observed disorganization of the cytoskeleton associated with disassembling of vinculin spots and phosphorylated proteins within focal contacts with no major change in TIRFM adhesion after 2 and 4 days of microgravity.*** Postmitotic cells, alone, accounted for the differences observed in the whole population. They are characterized by immature peripheral contacts with complete loss of central spots and decreased spreading. Osteocalcin, P1CP and alkaline phosphatase, and proliferation were similar in flight cells and 1 g centrifuge and ground controls after 6 days. In conclusion, microgravity substantially affected osteoblastic integrin-mediated cell adhesion. ROS17/2.8 cells responded differently, whether or not they were cycling by reorganizing adhesion plaque topography or morphology. In ROS 17/2.8, this reorganization did not impair osteoblastic phenotype. PMID: 11511518 [PubMed - indexed for MEDLINE] INE]

9) Exp Cell Res 1996 Apr 10;224(1):103-9

Effects of microgravity on osteoblast growth activation. Hughes-Fulford M, Lewis ML. Laboratory of Cell Growth and Differentiation, Department of Veterans Affairs, San Francisco, California, 94121, USA.

Space flight is an environmental condition where astronauts can lose up to 19% of weight-bearing bone during long duration missions. We used the MC3T3-E1 osteoblast to investigate bone cell growth in microgravity (10^{-6} to 10^{-9} g). Osteoblasts were launched on the STS-56 shuttle flight in a quiescent state with 0.5% fetal calf serum (FCS) medium and growth activation was initiated by adding fresh medium with 10% FCS during microgravity exposure. Four days after serum activation, the cells were fixed before return to normal Earth gravity. Ground controls were treated in parallel with the flight samples in identical equipment. On landing, cell number, cell cytoskeleton, glucose utilization, and prostaglandin synthesis in flight ($n = 4$) and ground controls ($n = 4$) were examined. *The flown osteoblasts grew slowly in microgravity with total cell number significantly reduced (55 +/- 6 vs 141 +/- 8 cells per microscopic field). The cytoskeleton of the flight osteoblasts had a reduced number of stress fibers and a unique abnormal morphology. Nuclei in the ground controls were large and round with punctate Hoechst staining of the DNA nucleosomes. The flight nuclei were 30% smaller than the controls ($P < 0.0001$) and oblong in shape, with fewer punctate areas. Due to their reduced numbers, the cells activated in microgravity used significantly less glucose than ground controls (80.2 +/- 0.7 vs 50.3 +/- 3.7 mg of glucose/dl remaining in the medium) and had reduced prostaglandin E2 (PGE2) synthesis when compared to controls (57.3 +/- 17 vs 138.3 +/- 41 pmol/ml).* Cell viability was normal since, on a per-cell basis, glucose use and prostaglandin synthesis were comparable for flight and ground samples. Taken together, these data suggest that growth activation in microgravity results in reduced growth, causing reduced glucose utilization and reduced prostaglandin synthesis, with significantly altered actin cytoskeleton in osteoblasts. PMID: 8612673 [PubMed - indexed for MEDLINE]

10) CRC Crit Rev Plant Sci 2000;19(6):551-73

Mechanisms of the early phases of plant gravitropism. Kiss JZ. Department of Botany, Miami University, Oxford, OH 45056, USA. kissjz@muohio.edu

Gravitropism is directed growth of a plant or plant organ in response to gravity and can be divided into the following temporal sequence: perception, transduction, and response. This article is a review of the research on the early events of gravitropism (i.e., phenomena associated with the perception and transduction phases). *The two major hypotheses for graviperception are the protoplast-pressure and starch-statolith models. While most researchers support the concept of statoliths, there are suggestions that plants have multiple mechanisms of perception.* Evidence supports the hypothesis that the actin cytoskeleton is involved in graviperception/transduction, but the details of these mechanisms remain elusive. A number of recent developments, such as increased use of the molecular genetic approach, magnetophoresis, and laser ablation, have facilitated research in graviperception and have allowed for refinement of the current models. In addition, the entire continuum of acceleration forces from hypo- to hyper-gravity have been useful in studying perception mechanisms. Future interdisciplinary molecular

approaches and the availability of sophisticated laboratories on the International Space Station should help to develop new insights into mechanisms of gravitropism in plants. PMID: 11806421 [PubMed - indexed for MEDLINE]

11) ASGSB Bull 1991 Jul;4(2):43-50

Gravity sensing mechanisms in plant cells. Sievers A. Botanisches Institut, Universitat Bonn, Germany.

Sensing of gravity is essential for the survival of plant seedlings. Therefore it is understandable that gravistimulation of only 0.5 sec-duration causes a graviresponse. The earliest graviresponses could be measured within seconds as alterations in membrane potentials of the statocytes in the root cap. Root statocytes are polarly organized. From a 6-day microgravity (10^{-3} - 10^{-4} g) experiment in the Spacelab D1 Mission it has been concluded that the observed polar differentiation is a result of a genetically prepatterned developmental program. Statoliths, the sedimentable organelles of statocytes, are surrounded by actin filaments which partly keep them in position. ***Under 6 min of microgravity during parabolic flights of rockets it could be demonstrated that the statoliths moved in the opposite direction to the initial gravity vector. It is concluded that shearing forces are exerted by microfilaments.*** It is supposed that the change of the position of statoliths is transmitted to gravisensitive structures of the statocytes (ER, plasma membrane) via microfilaments. As graviperception is influenced by calcium ions, it is suggested that these interactions regulate the activity of ion channels and/or pumps in the membranes thus initiating the graviresponse chain. ***In the case of cytoplasmic streaming in Chara rhizoids, the endogenous difference between the opposing streaming directions is diminished under microgravity during the flights of rockets. Possibly, shear stresses are affected by gravity, thus inducing gravity-related differences in the streaming velocities via actin filaments.*** PMID: 11537181 [PubMed - indexed for MEDLINE]

12) Adv Space Res 1999;24(6):755-62

Spaceflight experiments with Arabidopsis starch-deficient mutants support a statolith-based model for graviperception. Kiss JZ, Edelmann RE. Department of Botany, Miami University, Oxford, OH 45056, USA. kissjz@muohio.edu

In order to help resolve some of the controversy associated with ground-based research that has supported the starch-statolith theory of gravity perception in plants, we performed spaceflight experiments with Arabidopsis in Biorack during the January 1997 and May 1997 missions of the Space Shuttle. ***Seedlings of wild-type (WT) Arabidopsis, two reduced-starch strains, and a starchless mutant were grown in microgravity and then were given either a 30, 60, or 90 minute gravity stimulus on a centrifuge. By the 90 min 1-g stimulus, the WT exhibited the greatest magnitude of curvature and the starchless mutant exhibited the smallest curvature while the two reduced starch mutants had an intermediate magnitude of curvature.*** In addition, space-grown plants had two structural features that distinguished them from the controls: a greater number of root hairs and an anomalous hypocotyl hook structure. However, the morphological changes observed in the flight seedlings are likely to be due to the effects of ethylene present in the spacecraft. (Additional ground-based studies demonstrated that this level of

ethylene did not significantly affect gravitropism nor did it affect the relative gravitropic sensitivity among the four strains.) Nevertheless, this experiment on gravitropism was performed the "right way" in that brief gravitational stimuli were provided, and the seedlings were allowed to express the response without further gravity stimuli. Our spaceflight results support previous ground-based studies of these and other mutants since increasing amounts of starch correlated positively with increasing sensitivity to gravity. PMID: 11542619 [PubMed - indexed for MEDLINE]

13) 1: Int J Plant Sci 2001 Mar;162(2):249-55

Evidence of root zone hypoxia in Brassica rapa L. grown in microgravity. Stout SC, Porterfield DM, Briarty LG, Kuang A, Musgrave ME. Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA 70803, USA.

A series of experiments was conducted aboard the U.S. space shuttle and the Mir space station to evaluate microgravity-induced root zone hypoxia in rapid-cycling Brassica (Brassica rapa L.), using both root and foliar indicators of low-oxygen stress to the root zone. Root systems from two groups of plants 15 and 30 d after planting, grown in a phenolic foam nutrient delivery system on the shuttle (STS-87), were harvested and fixed for microscopy or frozen for enzyme assays immediately postflight or following a ground-based control. Activities of fermentative enzymes were measured as indicators of root zone hypoxia and metabolism. Following 16 d of microgravity, ADH (alcohol dehydrogenase) activity was increased in the spaceflight roots 47% and 475% in the 15-d-old and 30-d-old plants, respectively, relative to the ground control. Cytochemical localization showed ADH activity in only the root tips of the space-grown plants. Shoots from plants that were grown from seed in flight in a particulate medium on the Mir station were harvested at 13 d after planting and quick-frozen and stored in flight in a gaseous nitrogen freezer or chemically fixed in flight for subsequent microscopy. When compared to material from a high-fidelity ground control, concentrations of shoot sucrose and total soluble carbohydrate were significantly greater in the spaceflight treatment according to enzymatic carbohydrate analysis. Stereological analysis of micrographs of sections from leaf and cotyledon tissue fixed in flight and compared with ground controls indicated no changes in the volume of protoplast, cell wall, and intercellular space in parenchyma cells. Within the protoplasm, the volume occupied by starch was threefold higher in the spaceflight than in the ground control, with a concomitant decrease in vacuolar volume in the spaceflight treatment. Both induction of fermentative enzyme activity in roots and accumulation of carbohydrates in foliage have been repeatedly shown to occur in response to root zone oxygen deprivation. *These results indicate that root zone hypoxia is a persistent challenge in spaceflight plant growth experiments and may be caused by microgravity-induced changes in fluid and gas distribution*

14) J Appl Physiol 2002 Feb;92(2):817-25

Thin filament diversity and physiological properties of fast and slow fiber types in astronaut leg muscles. Riley DA, Bain JL, Thompson JL, Fitts RH, Widrick JJ, Trappe SW, Trappe TA, Costill DL. Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee 53226, USA. dariley@mcw.edu

Slow type I fibers in soleus and fast white (IIa/IIx, IIx), fast red (IIa), and slow red (I) fibers in gastrocnemius were examined electron microscopically and physiologically from pre- and postflight biopsies of four astronauts from the 17-day, Life and Microgravity Sciences Spacelab Shuttle Transport System-78 mission. At 2.5-microm sarcomere length, thick filament density is approximately 1,012 filaments/microm(2) in all fiber types and unchanged by spaceflight. ***In preflight aldehyde-fixed biopsies, gastrocnemius fibers possess higher percentages (approximately 23%) of short thin filaments than soleus (9%). In type I fibers, spaceflight increases short, thin filament content from 9 to 24% in soleus and from 26 to 31% in gastrocnemius. Thick and thin filament spacing is wider at short sarcomere lengths. The Z-band lattice is also expanded, except for soleus type I fibers with presumably stiffer Z bands.*** Thin filament packing density correlates directly with specific tension for gastrocnemius fibers but not soleus. Thin filament density is inversely related to shortening velocity in all fibers. Thin filament structural variation contributes to the functional diversity of normal and spaceflight-unloaded muscles. PMID: 11796697 [PubMed - indexed for MEDLINE]

15) Am J Bot 1987;74(2):218-23

Influence of microgravity on root-cap regeneration and the structure of columella cells in *Zea mays*. Moore R, McClelen CE, Fondren WM, Wang CL. Department of Biology, Baylor University, Waco, Texas 76798, USA.

We launched imbibed seeds and seedlings of *Zea mays* into outer space aboard the space shuttle Columbia to determine the influence of microgravity on 1) root-cap regeneration, and 2) the distribution of amyloplasts and endoplasmic reticulum (ER) in the putative statocytes (i.e., columella cells) of roots. ***Decapped roots grown on Earth completely regenerated their caps within 4.8 days after decapping, while those grown in microgravity did not regenerate caps. In Earth-grown seedlings, the ER was localized primarily along the periphery of columella cells, and amyloplasts sedimented in response to gravity to the lower sides of the cells. Seeds germinated on Earth and subsequently launched into outer space had a distribution of ER in columella cells similar to that of Earth-grown controls, but amyloplasts were distributed throughout the cells. Seeds germinated in outer space were characterized by the presence of spherical and ellipsoidal masses of ER and randomly distributed amyloplasts in their columella cells.*** These results indicate that 1) gravity is necessary for regeneration of the root cap, 2) columella cells can maintain their characteristic distribution of ER in microgravity only if they are exposed previously to gravity, and 3) gravity is necessary to distribute the ER in columella cells of this cultivar of *Z. mays*. PMID: 11539100 [PubMed - indexed for MEDLINE]

16) Neurosci Lett 2000 Dec 15;296(1):13-6

Weightlessness during spaceflight results in enhanced synapse formation in a fish brain vestibular nucleus. Ibsch M, Anken RH, Rahmann H. Zoological Institute, University of Stuttgart-Hohenheim, Garbenstrasse 30, D-70593, Stuttgart, Germany.

Synapse counts were undertaken by conventional electron microscopy in primary vestibular integration centers, (i.e. nucleus descendens and nucleus magnocellularis of the brainstem area octavolateralis) and in the diencephalic visual nucleus corticalis of spaceflown neonate swordtail fish *Xiphophorus helleri* as well as in 1 g control siblings. ***Spaceflight (16 days microgravity, (microg), STS-90 Neurolab Mission) yielded an increase in synaptic contacts within the vestibular nucleus descendens indicating that lack of input resulted in compensation processes.*** No effect of microg, however, was observed in the visual nucleus corticalis and in the vestibular nucleus magnocellularis which is situated in the close vicinity of the nucleus descendens. In contrast to the latter, the nucleus magnocellularis does not receive exclusively vestibular input, but inputs from the lateral line as well, possibly providing sufficient input at microgravity. PMID: 11099822 [PubMed - indexed for MEDLINE]

17) Physiologist 1985 Dec;28(6 Suppl):S93-4

Amphibian fertilization and development in microgravity.

Souza KA, Black SD. PMID: 3834503 [PubMed - indexed for MEDLINE]

18) Adv Space Res 1998;21(8-9):1155-8

Development of space-fertilized eggs and formation of primordial germ cells in the embryos of Medaka fish. Ijiri K. Radioisotope Center, University of Tokyo, Japan. In the second International Microgravity Laboratory (IML-2) mission in 1994, four small Japanese killifish (Medaka, *Oryzias latipes*) made a space travel of 15 days aboard a space shuttle. These four adult Medaka fish successfully mated in space for the first time among vertebrate animals. Moreover, the eggs they laid developed normally, at least in their external appearance, hatching as fry (baby fish) in space. Fish mated and laid eggs every day during the first week. Near the end of the mission most of the eggs had a well-developed body with two pigmented eyes. In total, 43 eggs were laid (detected), out of which 8 fry hatched in space, as truly 'space-originated' babies. A further 30 fry hatched within 3 days after landing. This is the normal hatching rate, compared with the ground-based data. Among the 8 space-originated fry, four were killed for histological sections, and germ cells at the gonadal region were counted for each fry. Their numbers were in the range of the germ cells of the normal control fry (ground-kept samples). ***Thus, as embryos developed normally in their external appearance, inside the embryos the formation of primordial germ cells took place normally in space, and their migration to the genital ridges was not hindered by microgravity.*** The two of the remaining space-originated fry have grown up and been creating their offspring in the laboratory. This proved that the primordial germ cells formed in space were also normal from a functional point of view. The four space-travelled adult fish re-started mating and laying eggs on the 7th day after landing and continued to do so every day afterward. ***Fertilization rate and hatchability of these eggs were as high as the eggs laid by the laboratory-kept fish. This fact implies that in gametogenesis of adult fish, there are no specific stages of germ cells extremely susceptible to microgravity.*** PMID: 11541366 [PubMed - indexed for MEDLINE]

19) Adv Space Res 1994;14(8):197-208

Fertilization of sea urchin eggs in space and subsequent development under normal conditions. Marthy HJ, Schatt P, Santella L. Observatoire Oceanologique, Universite P. & M. Curie, Banyuls-sur-mer, France.

Sea urchin eggs are generally considered as most suitable animal models for studying fertilization processes and embryonic development. In the present study, they are used for determining a possible role of gravity in fertilization and the establishment of egg polarity and the embryonic axis. For this purpose, eggs of the particularly well known and suitable species *Paracentrotus lividus* have been automatically fertilized under microgravity conditions during the Swedish sounding rocket flights MASER IV and MASER V. It turns out, that *fertilization "in Space" occurs normally and that subsequent embryonic and larval development of such eggs, continued on the ground, is normal, leading to advanced pluteus stages.* PMID: 11537918 [PubMed - indexed for MEDLINE]

20) Acta Astronaut 1981 Sep-Oct;8(9-10):995-1002

Hematological and immunological changes during space flight. Cogoli A. Laboratorium fur Biochemie, ETH-Zentrum, Zurich, Switzerland.

This paper gives a summary of the principal hematological and immunological changes observed in crews after space flight. Reduction of red blood cell mass (2-21%) and of hemoglobin mass (12-33%) is generally observed after the US and Soviet space missions. The changes are accompanied with a loss of plasma volume (4-16%). Erythrocyte and hemoglobin concentrations in the blood remain constant, suggesting that the changes are driven by a feed-back mechanism. *Immunological changes consist mainly of reduced T-lymphocyte reactivity. The results of the 96-day and 140-day Salyut-6 missions suggest that the adaptation of the immune system to spaceflight occurs in two stages: the first takes place during the first 2-3 months in space, the second follows and consists of further weakening of the immune response. Our experiments with human lymphocytes in vitro indicate that high-g enhance, whereas low-g depress lymphocyte activity.* Finally, our investigations to be performed on Spacelab are described.

21) Gravit Space Biol Bull 1997 Jun;10(2):5-16

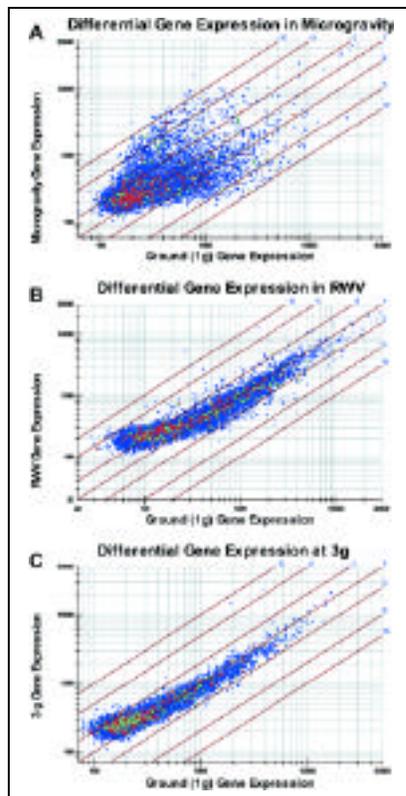
Signal transduction in T lymphocytes in microgravity. Cogoli A. Space Biology, ETH Technopark, Zurich, Switzerland.

More than 120 experiments conducted in space in the last 15 years have shown that dramatic changes are occurring in several types of single cells during their exposure to microgravity. One focus of today's research on cells in space is on signal transduction, especially those steps involving the cytoskeleton and cell-cell interactions. Signal transduction is often altered in microgravity as well as in hypergravity. This leads to changes in cell proliferation, genetic expression and differentiation. Interesting examples are leukocytes, HeLa cells, epidermoid cells and osteoblastic cells. *Signalling pathways were studied in T lymphocytes in microgravity by several investigators after the*

discovery that mitogenic activation in vitro is virtually nil at 0g. T cells are a good model to study signal transduction because three extracellular signals (mitogen, IL-1 and IL-2) are required for full activation, and two classical pathways (via proteins G and PKC) are activated within the cell. In addition, low molecular weight GTP-binding proteins (Ras and Rap) are interacting with the cytoskeleton. The data at 0g support the notion that the expression of IL-2 receptor is inhibited at 0g, while mitogen binding and the transmission of IL-1 by accessory cells occur normally. In addition, alterations of the cytoskeleton suggest that the interaction with Rap proteins is disturbed. Data obtained with phorbol esters indicate that the function of PKC is changed in microgravity. Similar conclusions are drawn from the results with epidermoid cells A431.

22) *Physiol Genomics* 2000 Sep 8;3(3):163-73

Mechanical culture conditions effect gene expression: gravity-induced changes on the space shuttle. Hammond TG, Benes E, O'Reilly KC, Wolf DA, Linnehan RM, Taher A, Kaysen JH, Allen PL, Goodwin TJ. Nephrology Section, Department of Medicine, Tulane/Veterans Affairs Environmental Astrobiology Center, New Orleans, Louisiana 70112, USA. thammond@mailhost.tcs.tulane.edu



Three-dimensional suspension culture is a gravity-limited phenomenon. The balancing forces necessary to keep the aggregates in suspension increase directly with aggregate size. This leads to a self-propagating cycle of cell damage by balancing forces. Cell culture in microgravity avoids this trade-off. We determined which genes mediate three-dimensional culture of cell and tissue aggregates in the low-shear stress, low-turbulent environment of actual microgravity. Primary cultures of human renal cortical cells were flown on the space shuttle. Cells grown in microgravity and ground-based controls were grown for 6 days and fixed. RNA was extracted, and automated gene array analysis of the expression of 10,000 genes was performed. *A select group of genes were regulated in microgravity. These 1,632 genes were independent of known shear stress response element-dependent genes and heat shock proteins. Specific transcription factors underwent large changes in microgravity including the Wilms' tumor zinc finger protein, and the vitamin D receptor. A specific group of genes, under the control of defined transcription factors, mediate three-dimensional suspension culture under*

microgravity conditions. PMID: 11015612 [PubMed - indexed for MEDLINE]

23) *FASEB J.* 15(10):1783-5. (2001)

cDNA microarray reveals altered cytoskeletal gene expression in space-flown leukemic T lymphocytes (Jurkat) Marian L. Lewis, Luis A. Cubano, Baiteng Zhao, Hong-Khanh Dinh, Jonathan G. Pabalan, Edward H. Piepmeier, and Phillip D. Bowman E-mail contact: lewisml@email.uah.edu

Cytoskeletal disruption and growth arrest consistently occur in space-flown human acute leukemic T cells (Jurkat). Although the microtubules appear to reorganize during spaceflight, cells remain nonproliferative. To test the hypothesis that spaceflight alters cytoskeletal gene expression and may thus affect cytoskeletal function, we flew Jurkat cells on Space Transportation System (STS) 95 and compared RNA message by cDNA microarray in space-flown vs. ground controls at 24 h (4,324 genes) and 48 h (>20,000 genes). *Messages for 11 cytoskeleton-related genes, including calponin, dynactin, tropomodulin, keratin 8, two myosins, an ankyrin EST, an actinlike protein, the cytoskeletal linker (plectin), and a centriole-associated protein (C-NAPI), were up-regulated in space-flown compared with ground control cells; gelsolin precursor was down-regulated. Up-regulation of plectin and C-NAPI message in both space-flown cells and vibrated controls is a novel finding and implies their role in vibration damage repair.* This first report of cDNA microarray screening of gene expression in space-flown leukemic T cells also identifies differential expression of genes that regulate growth, metabolism, signaltransduction, adhesion, transcription, apoptosis, and tumor suppression. *Based on differential expression of cytoskeletal genes, we conclude that centriole-centriole, membrane-cytoskeletal, and cytoskeletal filament associations are altered in the orbital phase of spaceflight.*

24) J Gravit Physiol 2000 Jan;7(1):S47-9

Effects of 14-day spaceflight on myosin heavy chain expression in biceps and triceps muscles of the rhesus monkey. Chopard A, Leclerc L, Pons F, Leger JJ, Marini JF. CNRS, Faculte des Sciences, Nice, France.

In rats, changes in myosin expression are induced by the chronic elimination of weight-bearing activity, particularly in the postural muscles. This occurs during spaceflight and hindlimb suspension. Myosin heavy chain (MHC) changes affect fast and slow fiber types differently depending on muscle function. An increase in co-expression of different MHC within the same fiber will signal early changes in muscle fibers. In the rat soleus muscles, the spaceflight-induced increase in fast MHC expression appears to be essentially due to the enhanced or de novo synthesis of IID or IIX MHC. *In response to microgravity, the expression of slow-type myosin decreases, while that of fast-type increases. There is scarce information concerning the effect of microgravity on rhesus monkeys (Macaca mulatta), especially on their upper limbs. We investigated the expression of MHC using an immunocytochemical approach to determine the nature and magnitude of the changes in biceps and triceps muscles of rhesus monkeys during the Bion 11 14-day mission.* PMID: 11543458 [PubMed - indexed for MEDLINE]