23

Gravitational Cell Biology

Cora S. Thiel and Oliver Ullrich

University of Zurich, Zurich, Switzerland

23.1 Gravitational Cell Biology

The evolution of life on Earth has been subject to a range of influences, whereas gravity was the only constant and universal force during all times of Earth's history. Since the last decades, a lot of evidence has been obtained suggesting that the function of mammalian cells and of small unicellular organisms is different under conditions of microgravity. Consequently, the question arose of how normal gravity may play a role in "normal" cellular function and if gravity may provide important signals for the cell. It is a common method to investigate the effects of forces by using systems in which these forces can be eliminated. In the case of gravity, this means that experimental environments have to be created where no or only minor gravitational forces prevail. A variety of platforms exist where these conditions can be achieved. Experiments can be performed under microgravity conditions of different length varying between seconds (drop tower or parabolic flights), minutes (sounding rockets) until up to permanent microgravity (ISS).

23.2 Studies Under Simulated Microgravity

However, the accessibility of these platforms is limited and cannot fully cover the needs of the research community. Therefore, platforms providing access to simulated microgravity are of great interest because they offer almost unlimited experimental capacity. The most frequently used ground-based facilities for these purposes (described in detail in Chapters 8 and 14) are the 2D clinostat, the random positioning machine (RPM), the rotating wall vessel (RWV), and the diamagnetic levitation [1]. These devices were not only used

for experiments with unicellular organisms such as Euglena, Paramecium, or Loxodes, but also for experiments with whole plants, animals, or plant and animal cell cultures. Simulated gravity studies with protists showed valuable results concerning their gravitaxis and gravikinesis [2, 3]. A comparison of the ground-based facilities used in experiments with the protists Euglena and Paramecium showed that simulated microgravity conditions generated by the fast rotating 2D clinostat are well suited, whereas the organisms experience a change in positive and negative acceleration forces when they are exposed to simulated microgravity on the RPM [1]. Experiments using magnetic levitation are less recommended, because of the strong effects of the magnetic field acting on these two types of protists [1, 4, 5].

23.3 Effects of Simulated Microgravity on Algae, Plant Cells, and Whole Plants

Simulated gravity is also used in experiments with algae, plant cell cultures, and whole plants. In Chara, the statolith-based gravity-sensing system in the rhizoids has been intensively investigated by 2D and 3D clinorotation as well as by magnetic levitation [6–8]. The comparison of the experimental outcomes showed that when Chara is used as a study object, the fast-rotating 2D clinostat is the preferred equipment to be applied for simulated microgravity, followed by the 3-D clinostat and the random positioning machine. In contrast, magnetic fields were not sufficient to generate the required simulated microgravity conditions [1].

Arabidopsis thaliana is a frequently used study object because of its excellent characterization on the molecular level. Gene expression analysis performed with whole plants on a 3D clinostat described the identification of new simulated microgravity stress-induced transcription factors [9]. Investigations performed with Arabidopsis cell cultures on 2D and 3D clinostats as well as under magnetic levitation showed altered gravity-related signaling cascades in the undifferentiated cultured cells [10].

23.4 Mammalian Cells in Simulated Microgravity

Mammalian cell cultures are frequently employed to study the direct effects of microgravity on the fundamental processes in the cells of our body. Cell lines, especially with fibroblast characteristics, are often used because of the easy handling and experiment preparations. It could be shown that under real and simulated microgravity conditions (2D clinostat or RPM), the epidermal

growth factor (EGF)-induced signaling pathway is affected and that cells changed their morphology to a roundish shape due to modifications affecting the cytoskeleton [11–13]. New experimental designs allow also for the use of the RPM to expose cells or even small organisms to simulated partial gravity [presented by Dutch Space (Leiden, The Netherlands) at the European Low Gravity Research Association in Vatican City in 2013 and in reference [14]].

Numerous comparative studies between real and simulated microgravity have also been performed with semi-adherent or non-adherent cells of the immune system. Semi-adherent NR8383 macrophages show a similar behavior under real and simulated microgravity conditions. The macrophageal oxidative burst reaction is one of the key functions in innate immune response. The associated phagocytosis-mediated reactive oxygen species (ROS) production is rapidly and reversely decreased upon reduced gravitation. The direct comparison between ground based and investigations under real microgravity during parabolic flights using a 2D clinostat combined with a one-axis clinostat fitted with a photo multiplier tube showed for both experimental setups a pronounced reduction in the ROS production and therefore of the reactivity of the cells [15]. Comparative studies have been also performed on U937 cells, a human myelomonocytic cell line. For both, real and simulated microgravity, a decreased proliferation rate was observed [16, 17]. Furthermore, a reduced locomotion was identified in monocytes most likely due to cytoskeletal modifications such as a reduced density of the actin filaments and impaired ß-tubulin architecture [18, 19]. T lymphocytes also strongly react to microgravity: They show an activation failure upon stimulation with ConA (for a review, see [20]). This effect was reproducible under simulated microgravity conditions in the RPM [21, 22]. In line with these results, stimulation of Jurkat T cells with PMA resulted in an increased expression of the cell cycle-regulating protein p21Waf1/Cip1 after 15min of 2D clinorotation. Real-time PCR experiments confirmed these results on gene expression level [23]. Importantly, differential gene expression studies from RPM experiments revealed that impaired induction of early genes regulated primarily by transcription factors NF-kappaB, CREB, ELK, AP-1, and STAT after T-cell receptor cross-linking contributed to T-cell dysfunction in altered gravity, associated with down-regulation of the PKA pathway [24].

Taken together, ground-based facilities for simulated microgravity are of great value and should be frequently used for preparation and/or validation of real microgravity experiments, where time and sample numbers are often limited. Several studies could show that 2D clinostats and the random positioning machine (RPM) are suitable to perform these ground-based simulation

experiments. However, during the experiment design phase, special attention shall be paid on choosing the most suitable system.

References

[1] Herranz, R., R. Anken, J. Boonstra, M. Braun, P.C.M. Christianen, M. de Geest, J. Hauslage, R. Hilbig, R. J.A. Hill, M. Lebert, F. J. Medina, N. Vagt, O. Ullrich, J. J.W.A. van Loon, and R. Hemmersbach. "Ground-Based Facilities for Simulation of Microgravity: Organism-Specific Recommendations for Their Use, and Recommended Terminology." *Astrobiology* 13 (2013): 1–17.

[2] Hemmersbach, R., and D.-P. Haeder. "Graviresponses of Certain Ciliates and Flagellates." *FASEB Journal* 13 (1999): S69–S75.

[3] Hemmersbach, R., S.M. Strauch, D. Seibt, M. Schuber. "Comparative Studies on Gravisensitive Protists on Ground (2D and 3D Clinostats) and in Microgravity. *Microgravity Science and Technology* XVIII-3/4 (2006): 257–259.

[4] Guevorkian, K., and J.M. Valles, Jr. "Aligning Paramecium Caudatum with Static Magnetic Fields." Biophysical Journal 90 (2006): 3004–3011.

[5] Guevorkian, K., J.M. Valles, Jr. "Swimming Paramecium in Magnetically Simulated Enhanced, Reduced, and Inverted Gravity Environments." *Proceedings of the National Academy of Sciences of the United States of America* 103 (2006): 13051–13056.

[6] Cai, W., M. Braun, and A. Sievers. "Displacement of statoliths in Chara rhizoids during horizontal rotation on clinostats." *Shi Yan Sheng Wu Xue Bao* 30 (1997): 147–155.

[7] Braun, M., B. Buchen, and A. Sievers. "Actomyosin-Mediated Statolith Positioning in Gravisensing Plant Cells Studied in Microgravity." *The Journal of Plant Growth Regulation* 21 (2002): 137–145.

[8] Limbach, C., J. Hauslage, C. Schafer, and M. Braun. "How to Activate a Plant Gravireceptor. Early Mechanisms of Gravity Sensing Studied in Characean Rhizoids During Parabolic Flights." *Plant Physiology* 139 (2005): 1030–1040.

[9] Soh, H., Y. Choi, T.-K. Lee, U.-D. Yeo, K. Han, C. Auh, and S. Lee. "Identification of Unique Cis-Element Pattern on Simulated Microgravity Treated Arabidopsis by in Silico and Gene Expression." *Advances in Space Research* 50 (2012): 397–407.

[10] Babbick, M., C. Dijkstra, O.J. Larkin, P. Anthony, M.R. Davey, J.B. Power, K.C. Lowe, M. Cogoli-Greuter, and R. Hampp. "Expression

of Transcription Factors After Short-Term Exposure of Arabidopsis Thaliana Cell Cultures to Hypergravity and Simulated Microgravity (2-D/3-D clinorotation, magnetic levitation). *Advances in Space Research* 39 (2007): 1182–1189.

[11] Boonstra. J. "Growth Factor-Induced Signal Transduction in Adherent Mammalian Cells is Sensitive to Gravity." *FASEB Journal* 13 (1999): S35–S42.

[12] Moes, M., J. Boonstra, and E. Regan-Klapisz. "Novel Role of cPLA(2)Alpha in Membrane and Actin Dynamics." *Cellular and Molecular Life Sciences* 67 (2010): 1547–1557.

[13] Pietsch, J., X. Ma, M. Wehland, G. Aleshcheva, A. Schwarzwälder, J. Segerer, M. Birlem, A. Horn, J. Bauer, M. Infanger, and D. Grimm. "Spheroid Formation of Human Thyroid Cancer Cells in an Automated Culturing System During the Shenzhou-8 Space mission." *Biomaterials* 34, no. 31 (2013): 7694–7705.

[14] Wuest, S.L., S. Richard, S. Kopp, D. Grimm, and M. Egli. "Simulated Microgravity: Critical Review on the Use of Random Positioning Machines for Mammalian Cell Culture." *Bio Med Research International*, 2015, Article ID 971474, (2014): 8 pp.

[15] Adrian, A., K. Schoppmann, J. Sromicki, S. Brungs, M. von der Wiesche, B. Hock, W. Kolanus, R. Hemmersbach, and O. Ullrich. "The Oxidative Burst Reaction in Mammalian Cells Depends on Gravity." *Cell Communication and Signaling* 11 (2013): 98.

[16] Hatton, J. P., F. Gaubert, M. L. Lewis, Y. Darsel, P. Ohlmann, J. P. Cazenave, and D. Schmitt. "The Kinetics of Translocation and Cellular Quantity of Protein Kinase C in Human Leukocytes are Modified During Spaceflight." *FASEB Journal* 13, (supplement) (1999): S23–S33.

[17] Villa, A., S. Versari, J. A.Maier, S. Bradamante. "Cell Behavior in Simulated Microgravity: A Comparison of Results Obtained with RWV and RPM." *Gravitational and Space Biology Bulletin* 18 no. 2 (2005): 89–90.

[18] Meloni, M.A., G. Galleri, P. Pippia, and M. Cogoli-Greuter. "Cytoskeleton Changes and Impaired Motility of Monocytes at Modelled Low Gravity." *Protoplasma* 229, no. 2–4 (2006): 243–249.

[19] Meloni, M.A., G. Galleri, G. Pani, A. Saba, P. Pippia, and M. Cogoli-Greuter. "Space Flight Affects Motility and Cytoskeletal Structures in Human Monocyte Cell Line J-111." *Cytoskeleton* 68, no. 2 (2011): 125–137.

[20] Cogoli-Greuter, M. "The Lymphocyte Story—An Overview of Selected Highlights on the In Vitro Activation of Human Lymphocytes in Space." *Microgravity Science and Technology*, 25, no. 6 (2014): 343–352.

[21] Walther, I., P. Pippia, M. A. Meloni, F. Turrini, F. Mannu, and A. Cogoli. "Simulated Microgravity Inhibits the Genetic Expression of Interleukin-2 and its Receptor in Mitogen-Activated T Lymphocytes." *FEBS Letters* 436, no. 1 (1998): 115–118.

[22] Schwarzenberg, M., P. Pippia, M.A. Meloni, G. Cossu, M. Cogoli-Greuter, and A. Cogoli. "Signal Transduction in T Lymphocytes—A Comparison of the Data from Space, the Free Fall Machine and the Random Positioning Machine." *Advances in Space Research* 24, no. 6 (1999), 793–800.

[23] Thiel, C.S., K. Paulsen, G. Bradacs, K. Lust, S. Tauber, C. Dumrese, A. Hilliger, K. Schoppmann, J. Biskup, N. Goelz,, C. Sang,U. Ziegler, K. H. Grote, F. Zipp, F. Zhuang, F. Engelmann., R. Hemmersbach, A. Cogoli, and O. Ullrich. "Rapid Alterations of Cell Cycle Control Proteins in Human T Lymphocytes in Microgravity." *Cell Communication and Signaling* 10 (2012): 1.

[24] Boonyaratanakornkit, J.B., A. Cogoli, C.F. Li, T. Schopper, P. Pippia, G. Galleri, M.A. Meloni, M. Hughes-Fulford. "Key Gravity-Sensitive Signaling Pathways Drive T Cell Activation." *FASEB Journal* 19, no. 14 (2005): 2020–2.