Why earth gravity is required for shaping life[*]

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Spaceflight technologies have disclosed amazing opportunities to outreach human knowledge and control over the natural World. However, the actual experience of microgravity has become a relevant threat that significantly limits the extent of man permanence in space. Since then, gravity effects on living organisms became a critical field of investigation. Gravity affects a wide array of biological functions, interacting at different levels of complexity, from molecules to cells, tissues and the organism as a whole. However, it is still a matter of investigation if gravity induces direct or indirect effects on cells. The non-equilibrium theory helps us in explaining how biological dissipative structures may be sensitive enough to sense gravity change, then transferring the mechano-signal into biochemical pathways. Within that framework, gravity represents an 'inescapable' constraint that obliges living beings to adopt only a few configurations among many others. By removing the gravitational field, living structures will be free to recover more degrees of freedom, thus acquiring new phenotypes and new properties. Discoveries on that field are supposed to advance our knowledge, providing amazing insights into the biological mechanism underlying physiology as well as many relevant diseases.

Our studies, carried out in the Systems Biology Group Lab (Sapienza University, Rome, www.sbglab.org) have investigated cells and tissues growing both in simulated microgravity on Earth (through the Random Positioning Machine) as well as in real weightlessness (onboard of the International Space Station, directly performed by the astronaut Lt. Colonel Samantha Cristoforetti). Investigation focused on biochemical pathways and cell structures (shape, cytoskeleton), involved several high-throughput technologies, including PCR, transcriptome assay, western-blot, HPLC/MS, confocal and electronic microscopy. Studies were conducted on both animal and human cells/tissues, including normal and cancerous samples. Phenotypic transition enacted by loss of gravity constraints has been carefully monitored across different times, and it has been described by means of a mathematical modelling. Our latest study is currently in press on Nature microgravity ("Phenotypic transitions enacted by microgravity do not alter coherence in gene transcription profile").

The impact of gravity on life

We are so acquainted with the daily experience of gravity, that we can hardly wonder life without it. Every day experiences are so inextricably embedded into the gravitational field that even our neuro-physiological cognitive processes have been molded into a specific neuronal network configuration, i.e. an internal model of gravity to cope with.[1] By considering gravity as an unavoidable *conditio sine qua non*, we have been ultimately unable in appreciating gravitational influences on life or even we assumed that gravity irrelevant in living processes, as it was actually impossible neither to rule out experiment in a modified gravitational field, not to detect any relevant change in cells or other living structures.

However, the development of space technologies, opening to humankind the adventure of space flight, makes this issue more than a theoretical one. How gravity could modify normal human physiology, or induce damages, eventually leading to a true pathological condition, thus became a critical field of investigation to ensure safest health conditions during space missions.

Indeed, research on space microgravity is often rationalized by claiming that it may/should improve the health of astronauts or the understanding of degenerative diseases (osteoporosis, muscle atrophy, cancer).

However, the common motivating cue underlying attempts to evaluate biological phenomena as they occur on a microgravity field is definitely the theoretical aspiration to understand the role that a true physical factor (gravity) can plays in shaping life. Gravity, even constant throughout the history of Earth, constitutes a true *evolutionary force*, i.e. a boundary constraint to which evolving organisms have to cope with in order to face the selective environmental pressure, getting better chances of success.

Microgravity effects on living organisms

Galilei first guessed Earth's gravity influence on living structures and processes. Making measurements made from bones of animals of different weights, he was able to observe that the length-to-width ratio diverges significantly among light and heavy animals.[2] Yet, it was not until 1985 that the dramatic impact of gravity on living systems was convincingly confirmed by experiments made both on ground and on the Spacelab D-1 flight, thus overcoming the bias represented by the lack of proper controls.[3] Since then, several reports pointed out living cells in microgravity field show major changes, involving metabolism, cytoskeleton, membrane structure, gene regulation, shape and many other biological properties.[4]

Direct microgravity effects on cells

At a cellular or sub-cellular level, the "force" of gravity is apparently insignificant compared to the three other basic forces in nature: the weak nuclear force, the strong nuclear force and the electrostatic force, that govern the force field within and between molecules inside a cell. Furthermore, non-gravity related phenomena like thermal noise (*kT*) or chemical energies are orders of magnitude larger than 1*g* acceleration.

However, an impressive set of scientific data demonstrated changes in gravitational field (i.e., hypo-gravity) are responsible of dramatic effects on living organism. Gravity-related effects can be observed at different level of observations: from molecular dynamics to physiologic complex functions, even after few minutes of exposure.[5] These data made a serious impact on the current thinking about the significance of gravity in basic living processes, and a different conceptual approach is required in order to provide reliable models of interpretation. Meanwhile overall macroscopic effects of gravity on physiologic functions are readily understandable by means of classic (i.e., "Newtonian") science, there is not yet a comprehensive theoretical framework able to explain on principle grounds how individual cells may sense gravity and respond to its changes.

For long time it has been supposed gravity not to influence the cellular machinery directly, but to exert its effect on the bulk volume of the surrounding medium. Diffusion and convention flow are both gravity-dependent processes and they support the proper influx of nutrients as well as the efflux of metabolic products at the cellular boundary. Microgravity reduces fluid shear stress, convection processes and chemical diffusion; in addition, some physical properties–like surface tension–of living cells are directly hindered by gravitational acceleration.

However, it makes an enormous scientific difference if cell functions by their own would be sensitive to gravity, than if cells simply react to a changed chemical environment. Therefore, one may ask how cells can sense gravity. Indeed, with some exceptions (plant root statocytes) in animal cell do not exist molecular complexes adapted for gravity sensing. Nevertheless, even if the force exerted by gravity inside the cell is of order of magnitude weaker than molecular forces, some mechanisms–mainly explained by non-equilibrium thermodynamics–can amplify the relatively weak signals to above the level of the thermal noise, as pointed out by the seminal work of Prigogine.[6]

Non-equilibrium processes

The state of equilibrium is the state of maximum entropy, i.e. the maximum disorder. In this condition there is no cooperativity in the system, except near a phase transition. Hence, the influence of an external field–whatsoever its nature–on such a system is characterized by the ratio of energy interaction (with the field) and the thermal energy of the molecule.[7] Considering a gas whose molecules have a masse *m*, enclosed in a volume of length *l*, a gravitational field of strength *g*, the maximum energy of interaction is (1) $W = mgl$.[a] The ratio mgl/kT is extremely small (<1) for value of *l* of the order of centimetres. Therefore, the field can actually produce a quantitative effect only when $mgl > kT$, when the field potential is larger than the thermal fluctuations (kT). This happens when there is cooperativity between the molecules in bringing about a macroscopic order: the effect of the field "adds up" to produce a large effect. In equilibrium systems this occurs during a phase transition; in non-equilibrium systems the same phenomenon is more frequent to happen, due to richness of instabilities and the consequent transition to very intricate dissipative structures. In these conditions, transition from a homogenous to an inhomogeneous stationary patterns can occur in classic reaction-diffusion processes, in which the behavior of the concentrations of reacting chemicals is the described by the equation (2): $D\nabla^2 C + F(C,\lambda) = \delta C/\delta t$ + boundary conditions.[b] This relationship implies that non-linear systems, when sufficiently far from equilibrium, can form different spatial structures and evolve into different attractors. Indeed, when λ is less than the critical value of λc, the concentration ck are homogeneous, but when $\lambda > \lambda_c$ the homogeneous state becomes unstable and the system can evolve into one of several possible states (Fig. 1).[c]

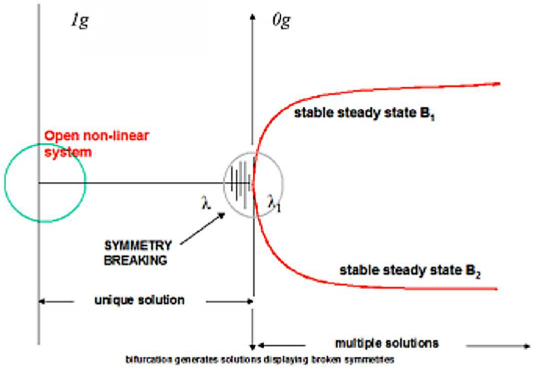

Fig.1. The system begins with an initial value of parameter λ. When λ increases to λ1, the system enters a non-equilibrium state that leads to symmetry breaking and to the emergence of bi-stability. Thereafter, a new ordered behavior emerges, characterized by multiple choices and different stable states, characterized, for g~0, by an equal likelihood.

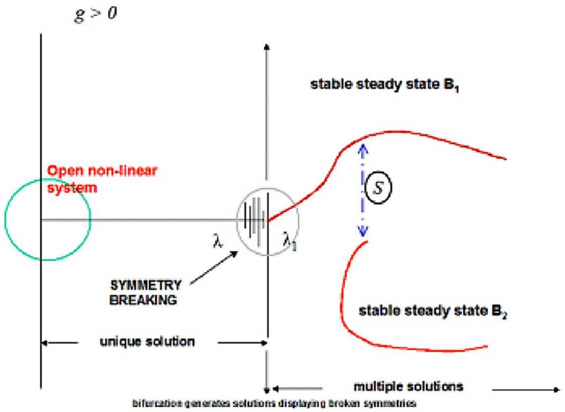

Fig.2. After the bifurcation, in presence of a gravitational field (1*g*) the system is preferentially committed toward the stable state B1. This means that the two attractors (B1 and B2) do not possess the same probability to occur. The parameter separation S is proportional to $(mgl/kT)^{1/3}$. The parameter S is a reliable measure of the system sensitivity to the field strength.

Transition to such states is mathematically formalized by bifurcating solutions to the equation (2); if such a system is coupled to an external field, the symmetry is broken. Now a directionality emerges and consequently the system may evolve into one of the state and not the other. For a field (gravitational, electromagnetic, morphogenetic) the effectiveness with which the system is committed into one of the states is a measure of the sensitivity of the system. The equation (2) assumes the form of: $D\nabla^2 C - \mu g \times \nabla C + F(C,\lambda) = \delta C/\delta t$ + boundary conditions (3), where *g* is the external gravitational field. It can be demonstrated that solution to the equation (3) assumes the form of: $B\alpha^3 + A_1\lambda\alpha + A_2 g = 0$ (Eq. 4). For a non zero *g* the two branches are separated, but if the bifurcation parameter is changed, the system will evolve preferentially evolve into one of the branches and not the other, unless there is a large enough internal fluctuation or an external perturbation. By this way the field select one of the two possible structures and the minimum separation S (Fig.2) is a measure of the potency of the field. Clearly, S is dependent on the strength of the field. When the field strength is reduced (as happens for instance when g is below the earth reference value, i.e. 9.8 cm sec^{-1}), thermal fluctuations will make the separation unobservable. It can be demonstrated that, for non-equilibrium systems $S = \frac{3}{2}(\frac{g\xi}{kT}\frac{l}{(n_c\pi)^2})^{1/3}\zeta$ (Eq. 5). Unlike $(\frac{g\xi}{kT})$ is a small number, its value is enhanced due to the exponent 1/3. It has been demonstrated that in non-equilibrium systems sensitivity to field's strength is about six order of magnitude larger than response in equilibrium systems. This result is amazing because it can provide affordable response to the unexpected sensitivity of dissipative systems to apparently weak fields. It is noteworthy that the concentration variation of the non-equilibrium system is of the same magnitude as S. Therefore, the bifurcation process is enhanced by a factor of 10^6. Since S depends on $g^{1/3}$, if we reduce the strength of g by a factor of 10^6, then S will be reduced by a factor of 10^2. This result implies that in microgravity sensitivity of non-equilibrium systems is highly decreased (from 10^6 to 10^2). Hence, reaching two possible states will be almost equally probable. In other words, normal gravity significantly contributes to drive non equilibrium systems to a preferential attractor, meanwhile, in microgravity, different dissipative structures are equally likely to occur. From a different perspective, a reduced gravitational field allows the system to reach several different states with the same likelihood: more attractors will be accessible and therefore the overall entropy will increase. In other words, in normal *g* conditions, gravity produces order and contributes to the commitment of the system towards a discrete number of stable states (attractors). Such theoretical framework has been vindicated by experimental results. Indeed, different human cell types cultured in microgravity undergo dramatic morphology changes, leading to two alternative phenotypes: an 'adherent' and a 'clumps-organoid' one, simultaneously present in the same culture.[8,9,10](Fig.3).

This is reversible process: when a 'clumps-organoid' population is growing in normal gravity, it goes back to the usual phenotype and, when re-seeded in microgravity condition, it gives rise again to the two above-mentioned phenotypes.

The overall meaning of such findings is that in absence of gravity-dependent constraints the system is unable to choose in between two different phenotypes, thus leading cells to be partitioned into two different clusters. In other words, the genotype does not determine by itself the phenotype but require additional, environmental cues to proper finalize cell differentiation.[11] In fact, absence of (physical) constraint impairs proper differentiation and eventually enacts

Fig. 3

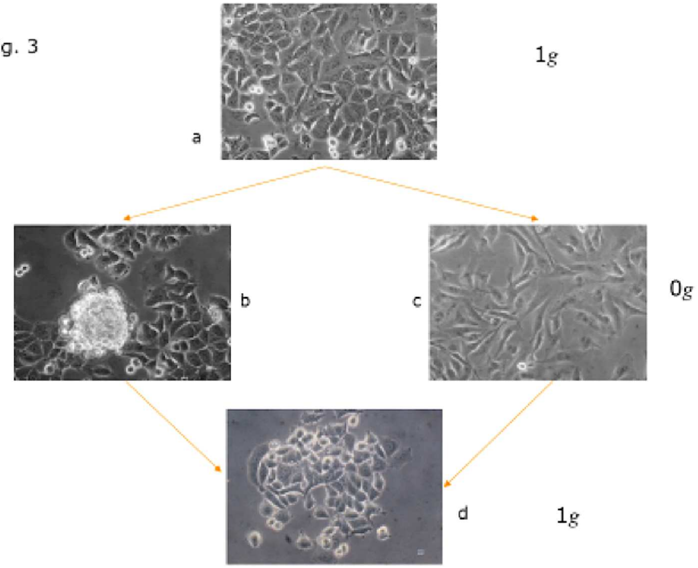

Fig.3. Phenotypic changes in cells exposed to different gravity conditions. MCF7 cells cultured under static 1g-conditions grew as a normal 2D monolayer (Fig. 3a). MCF7 cells in microgravity resulted partitioned into two phenotypes. The first, represented by floating-clump cells, and the second constituted by adherent cells (Fig. 3b,c). Both phenotypes revert to the native morphology when they are reseeded in normal gravity, independently from the time they have spent in microgravity (Fig. 3d, from ref. in note 8, modified)

opposite effects on different cell clusters. In absence of gravity, the correct developmental/differentiation pathways leading to an organized individual are therefore severely disturbed and hindered.[12] These results cast on doubt that normal embryological and developmental life processes could take place in absence of Earth gravity.

The future is near

Gravity has constantly influenced both physical as well as biological phenomena throughout all the Earth's history. The gravitational field has probably played a major role in shaping evolution when life moved from water to land, even if, for a while, it has been generally deemed to influence natural selection only by limiting the range of acceptable body sizes, according to Galilei's principle. Indeed, to counteract gravity, living organisms would need to develop systems to provide cell membrane rigidity, fluid flow regulation, and appropriate structural support and locomotion. However, gravity may influence in a more deep and subtle fashion the way the cells behave and build themselves. Gravity, indeed, represents an 'inescapable' constraint[13] that obliges living beings to adopt only a few configurations ('attractors') among many others. By removing the gravitational field, living structures will be free to recover more degrees of freedom, thus acquiring new phenotypes and (probably) new functions/properties.[14] That statement raises several critical questions. Some of these entail fundamentals of theoretical biology, as they cast doubt on the classical molecular paradigm on which modern biology has been build:[15] therefore, it can be argued that the ultimate reason for human space exploration is precisely to enable us to discover ourselves. Undoubtedly, the microgravitational-space field presents an unlimited horizon for investigation and discovery. Controlled studies conducted in microgravity can advance our knowledge, providing amazing insights into the biological mechanism underlying physiology as well as many relevant diseases, like cancer.[16] Accordingly, space-based investigations may serve as a novel paradigm for innovation in basic and applied science.

Others topics have a clear medical and practical relevance, as they are closely tied to the actual possibility to afford human permanence in space. How much gravity is needed to maintain life as we know it? Are the Moon's and Mars's gravity sufficient enough to ensure the minimum 'gravity loading' required to ensure that goal?, that is to say there exist a threshold gravitational level? How life could be assured in the outer space ($10^{-6}g$ or lower values), and, eventually how could living organisms evolve?

Arguably, future research will be committed to provide a satisfactory answer to those crucial problems, if we are to travel and to live in the space environment.

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[1] M, Zago & F. Lacquaniti, "Internal model of gravity for hand interception: parametric adaptation to zero-gravity visual targets on Earth", *Journal of Neurophysiology* 94(2), 2005, 1346-1357.

[2] G. Galilei, *Two New Sciences: Including Centers of Gravity and Force of Percussion*, trans. S. Drake, Toronto, Wall & Thompson, 1974, 109-146

[3] A. Cogoli (ed.), *Cell Biology and Biotechnology in Space (Advances Space Biology and Medicine, 8*), Amsterdam, Elsevier Science, 2002.

[4] P. Todd, D. M. Klaus, S. Stodieck, J. D. Smith, A. Staehelin, M. Kacena, B. Manfredi & A. Bukhari, "Cellular responses to gravity: Extracellular, intracellular and in-between", *Advances in Space Research* 21(8-9), 1998, 1263-1268.

[5] R. J. White & M. Averner, "Humans in space", *Nature* 409(6823), 2001, 1115-1118.

[6] D. K. Kondepudi & I. Prigogine, "Sensitivity on nonequilibrium chemical systems to gravitational field", *Advances in Space Research* 3(5), 1983, 171-176

[7] D. K. Kondepudi & I. Prigogine, "Sensitivity of non-equilibrium systems", *Physica A: Statistical Mechanics and its Applications* 107, 1981,1-24.

[8] M. G. Masiello, A. Cucina, S. Proietti, A. Palombo, P. Coluccia, F. D'Anselmi, S. Dinicola, A. Pasqualato, V. Morini & M. Bizzarri, "Phenotypic Switch Induced by Simulated Microgravity on MDA-MB-231 Breast Cancer Cells", *BioMed Research International* 2014, 652434

[9] S. Kopp, L. Slumstrup, T. J. Corydon, J. Sahana , G. Aleshcheva, T. Islam, N.E. Magnusson, M. Wehland, J. Bauer, M. Infanger & D. Grimm, "Identifications of novel mechanisms in breast cancer cells involving duct-like multicellular spheroid formation after exposure to the Random Positioning Machine", *Scientific Reports* 6, 2016, 26887.

[10] D. Grimm, M. Wehland, J. Pietsch, G. Aleshcheva, P. Wise, J. van Loon, C. Ulbrich, N.E. Magnusson, M. Infanger & J. Bauer "Growing Tissues in Real and Simulated Microgravity: New Methods for Tissue Engineering", *Tissue engineering Pt. B Reviews* 20(6), 555-566.

[11] M. G. Masiello, R. Verna, A. Cucina, M. Bizzarri, "Physical constraints in cell fate specification. A case in point: Microgravity and phenotypes differentiation", *Progress in Biophysics and Molecular Biology* 134, 2018, 55-67.

[12] Y. Wang, Y. Xie, D. Wygle, H. H. Shen, E. E. Puscheck & D. A. Rappolee, "A Major Effect of Simulated Microgravity on Several Stages of Preimplantation Mouse Development is Lethality Associated With Elevated Phosphorylated SAPK/JNK", *Reproductive sciences* 16(10), 2009, 947-59.

[13] G. Longo, M. Montevil, *Perspectives on Organisms: Biological Time, Symmetries and Singularities*, Berlin, Springer, 2014.

[14] M. G. Masiello, R. Verna, A. Cucina, M. Bizzarri, "Physical constraints in cell fate specification, *op. cit.*

[15] M. Bizzarri, A. Palombo, A. Cucina, "Theoretical aspects of Systems Biology", *Progress in Biophysics and Molecular Biology* 112, 2013, 33-43.

[16] J. L. Becker & G. R. Souza, "Using space-based investigations to inform cancer research on Earth", *Nature Reviews Cancer* 13, 2013, 315-327.

a The equation (1) has the characteristic of a potential and represent the work the field can produce on particle of masse m, displacing it along a length l.

b D is the diffusion matrix; $C = (c_1, c_2,\ldots c_n)$ is the n-vector representing the concentrations of the n reacting chemicals; F is a non linear operator expressing the chemical kinetics; λ is a parameter representing the constraints on the system that keep it away from equilibrium.

c For the sake of simplicity in Fig. 1 we have represented only two possible states. Each of the two states are asymmetric about the centre and are mirror reflections of each other. The two states need not to have this particular symmetry: their symmetry about the centre depends on the critical wave number n_c, associated with λ_c