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Microgravity environment uncouples cell growth and cell proliferation in root meristematic cells

The mediator role of auxin

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Experiments performed in space have evidenced that, in root meristematic cells, the absence of gravity results in the uncoupling of cell growth and cell proliferation, two essential cellular functions that support plant growth and development, which are strictly coordinated under normal ground gravity conditions. In space, cell proliferation appears enhanced whereas cell growth is depleted. Since coordination of cell growth and proliferation is a major feature of meristematic cells, the observed uncoupling is a serious stress condition for these cells producing important alterations in the developmental pattern of the plant. Auxin plays a major role in these processes both by assuring the coupling of cell growth and proliferation under normal conditions and by exerting a decisive influence in the uncoupling under altered gravity conditions. Auxin is a mediator of the transduction of the gravitropic signal and its distribution in the root is altered subsequent to a change in the gravity conditions. This altered distribution may produce changes in the expression of specific growth coordinators leading to the alteration of cell cycle and protein synthesis. Therefore, available data indicate that the effects of altered gravity on cell growth and proliferation are the consequence of the transduction of the gravitropic signal perceived by columella cells, in the root tip.

The size and morphology of plants and of plant organs is basically determined by cellular activities that occur in meristems. The primary meristems are root and shoot apical meristems, located at both upper and lower ends of the plant, which are constituted by stem cells. Cell division in these meristems is required to supply new cells for expansion and differentiation of tissues and initiation of new organs, providing the basic structure of the plant body.[1] In turn, active protein synthesis is required after mitosis in order to promote the necessary cell growth, up to duplication of cell size, which will make possible a new cell division. This continuous activity of growth and proliferation in meristematic cells is controlled by auxin, whose distribution in roots sets up distinct zones for cell division, cell expansion and differentiation and determines the balance between them.[2,3]

Therefore, cell growth and proliferation are essential functions for plant development and they are involved in the developmental response to environmental stimuli, such as tropisms and defense mechanisms against both biotic and abiotic agents.[4-6] Gravity is a fundamental environmental condition, constant in the Earth as a factor conditioning life throughout its whole history. Plants are particularly affected by gravity in their growth, which is directed by the gravity vector producing the well known process of gravitropism.

An experiment aimed to know the effects of a weightless environment on cell proliferation and growth in root meristematic cells was performed in the International Space Station. It consisted of germinating seeds of *Arabidopsis* *thaliana* in space and then growing seedlings for four days at the constant temperature of 22°C, in the darkness. Seedlings were fixed when still in space and recovered on ground to be processed for microscopical study. In addition, samples from a previous space experiment, grown in a similar way but fixed differently and including a control flight experiment in a 1 *g* centrifuge, were also incorporated to the analysis.[7,8] This analysis consisted of biometrical estimations of the seedling and root length, quantitative measurements at the cellular level, including number of cells per millimeter in specific cell files, in order to get an estimate of the cell proliferation rate, and morphometrical, ultrastructural and immunocytochemical study of the nucleolus, in order to know the rate of ribosome biogenesis, as an estimation of the level of protein synthesis, which is the cellular process which determines cell growth in the root meristem. Data obtained from space-flown samples were compared with 1 *g* ground controls and also with data from samples grown in the same conditions in a device called "Random Positioning Machine", an efficient simulator of microgravity, which induces constant changes of the gravity vector as it is sensed by living samples.[9] The results interestingly showed an enhanced rate of cell proliferation accompanied by a reduction of ribosome biogenesis per cell in samples grown in both real and simulated microgravity, compared to 1 *g* controls, either in flight or on ground.[10] This alteration of essential cellular processes may go far beyond the mere change in specific physiological activities of a particular cell type, since, on the one hand, alteration of cell growth and proliferation in the root meristem may have consequences at the level of development and shaping of the whole plant; on the other hand, regulation of these cellular activities by auxin may put in connection these cellular alterations with the transduction cascade of the gravitropic signal perceived by columella cells in the root tip, which is altered when the environmental gravity conditions change and which finally results in the modification of the levels and distribution of auxin throughout the root.

The Strict Link Between Cell Growth and Cell Proliferation is a Major Feature of Plant Meristematic Cells, so the Uncoupling of these Two Cellular Processes Caused by Microgravity Conditions is a Major Stress for these Cells

In meristematic cells, growth and proliferation are strictly coupled so that growth is required for proliferation to produce daughter cells whose size is above the threshold of biomass that allows cell life. Nevertheless, both cell activities have their own independent mechanisms of regulation. In the literature, we can find examples of physical treatments and/or induced expression of certain genes leading to either block of proliferation, but not of growth (resulting in larger cells), or to enhancement of proliferation, but not of growth (resulting in larger numbers of smaller cells).[11-13] The consequences of either of these two alterations are detrimental, if not deleterious for the plant; thus, it is essential a correct coupling of these two activities and of their mechanisms of regulation to determining a correct developmental program leading to adequate sizes of the organs and of the whole plant.

Whereas the concept of cell proliferation is unambiguous in reference to cell division, the concept of cell growth may be confusing and is worth to be clarified. Plant cells may grow either via increase in its cytoplasmic mass or via expansion of intracellular vacuoles. Vacuolar expansion is a feature of cell differentiation and accounts for the growth of differentiated organs. On the contrary, cytoplasmic growth is an attribute of rapidly cycling cells, such as meristematic cells, in which vacuoles are extremely small.[14] Cytoplasmic growth accompanies the increase of size of the cell nucleus, which occurs throughout the interphase of the cell cycle and it is essentially due to the activity of protein synthesis.[13] Therefore, cytoplasmic growth is strictly correlated to the rate of biogenesis of ribosomes, the factories of proteins. Furthermore, the increase in the population of ribosomes is an indication of cell growth in meristematic cells, since this population must be shared out after mitosis between the two daughter cells.

The rate of ribosome biogenesis can be accurately estimated by some parameters reporting certain features of the molecular cytology of the nucleolus. It has been repeatedly demonstrated that, in actively proliferating cells, the nucleolar size, the proportion and topological distribution of the nucleolar granular component and the number, size and structure of nucleolar fibrillar centers are strictly associated to the rate of transcription and processing of ribosomal precursors.[15,16]

In addition to the independent mechanisms of regulation of cell proliferation ad growth, there are coordinators that function in the maintenance of the coupling of the two processes. One example is the *AINTEGUMENTA (ANT)* gene, which regulates organogenesis by maintaining ongoing cell proliferation coordinately with cell growth, i.e., the classically called "meristematic competence", until a certain size of the organ is reached.[13] Other cases, maybe more complex, are those of proteins whose activity is regulated by signals coming from both cell cycle and protein synthesis agents. An example of this is nucleolin, a multifunctional protein located in the nucleolus whose function leads to activation of different steps of pre-rRNA synthesis and processing during the process of biogenesis of ribosomes.[16] This activity of nucleolin in modulating cell growth is regulated by phosphorylation by casein kinase II and CDKB, known agents of regulation of cell proliferation and cell cycle.[17-19] These examples are only a few known cases of what should be a complex mechanisms operating at multiple levels, whose full discrimination is only beginning to be known at the present time. Actually, the molecular nature of meristematic competence is presently unknown.[13]

Therefore, it appears to be clear that uncoupling of cell proliferation and cell growth in root meristematic cells requires disrupting a network of interactions and unbalancing a delicate equilibrium of activators and repressors. Undoubtedly, this must be the response to a situation of serious alteration of the environmental conditions. Therefore, if the suppression of the gravity vector is capable of producing such these effects, it results that gravity is essential for sustaining a normal bodyplan and weightlessness is a major stress condition for plant development and growth.

The Alterations Observed in Root Meristematic Cells: Are they a Consequence of the Transduction of the Gravitropic Stimulus from Columella Cells, or Do they Respond to a Specific Gravistimulus Perceived Directly by Meristematic Cells?

It is known that the gravity vector can be sensed by cells as a mechanical signal, which is capable of being transduced. In plants, it is well known that specialized cells located in the root tip—statocytes—perceive changes in gravity in the form of a differential sedimentation of starch particles—statoliths. There exists a cascade of signals which transmits the physical information derived from statolith sedimentation to the cells of the elongation zone, throughout the root meristem.[20,21] Otherwise, in both plant and animal cells it has been reported the perception of mechanical signals by cells not apparently specialized in gravity sensing.[22-24]

Transduction of the mechanosignal produces the reorientation of auxin efflux carriers and the subsequent redistribution of auxin streams in the root. Under normal environmental conditions, auxin is transported basipetally, throughout peripheral tissues of the root, up to the elongation zone by means of specific auxin efflux and influx carrier proteins.[3,25] Growth in microgravity (real or simulated) results in a substantial inhibition of the auxin polar transport;[26,27] otherwise, when auxin transport was experimentally inhibited, the gravitropic response was suppressed.[28]

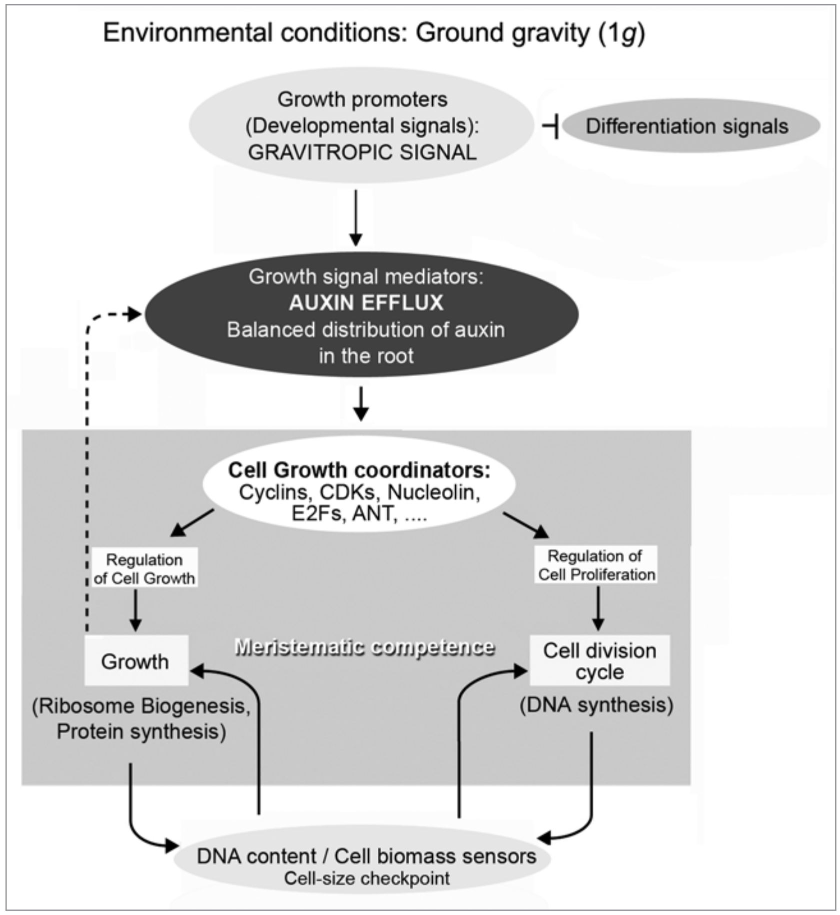

Figure 1. Model of the actions and interactions leading to the maintenance of the meristematic competence (involving the coordination between cell growth and cell proliferation) specifically showing the players involved in the transduction of the gravitropic signal. Under normal ground gravity conditions, this gravitropic signal is a growth promoter, since it stimulates the growth of the primary root according to the gravity vector. The mechanic stimulus is transduced by auxin, which is the mediator that, in the particular case of meristematic cells, modulates the expression of a variety of molecules (cyclins, CDKs, nucleolar proteins, transcription factors such as E2Fs, secondary mediators such as ANT, etc.,) that coordinate the regulation of cell growth and cell proliferation and finally keep coupled the processes of protein synthesis (cell growth) and cell cycle progression (cell proliferation). A change in the gravity conditions disrupts this equilibrium leading to the dis-coordination or uncoupling of the two essential cellular processes, and to the severe alteration of the meristematic competence. Scheme adapted and modified from Mizukami.[13]

Auxin is a phytohormone that influences multiple aspects of growth and differentiation in plants, among which the coordination between cell growth and cell division. Low levels of auxin induce cell elongation, enlargement and differentiation, whereas high levels of auxin stimulate cell proliferation and cell cycle progression. Different targets of auxin, directly acting on the mechanisms of cell cycle and cell growth have been described; one of them, the transcription factor E2FB, when expressed constitutively, is capable of reproducing the same phenotype as described for the weightless environment, i.e., elevated cell numbers and small cell sizes, in other words, the uncoupling of cell growth and cell proliferation.[14] These effects are associated with a shortening of the cell cycle, caused by the accelerated entry in the S phase from G_1, and in mitosis from G_2. This and other targets of auxin acting in a similar way,[29] combined with the alteration of the auxin distribution in the root as an effect of the gravity modification, strongly suggest that the role of auxin as a mediator of the maintenance of coupling of cell growth and proliferation under normal gravity conditions is modified as a consequence of the change in the environmental gravity conditions (**Fig. 1**).

Future Prospects

Since an experiment relatively simple in its setup (although with the complexity inherent to space experiments characterized by multiple specific constraints) has led to the finding that the microgravity environment is a serious stress condition for root meristematic cells, it is now necessary to characterize in detail the particular mechanism of the alteration by using new and more powerful methods and techniques. It will be necessary to characterize specific genes whose expression may be enhanced or depleted in these conditions; furthermore, the comparison of cell cycle features in experimental and control conditions will be highly informative. Finally, the specific pattern of auxin distribution under experimental conditions needs to be defined in wild type samples, as well as in different mutants affecting to auxin responsive elements. For these purposes, the analysis of the root meristem may become insufficient in some cases, since it may not be able to produce enough material as it is necessary to perform genomic or proteomic techniques specifically on proliferating cells. For this reason we will approach the use of in vitro cell cultures as large homogeneous populations of proliferating cells, which will provide us with new data, decisive for the understanding of the alterations studied. Experiments are currently in progress, using ground-based facilities simulating microgravity, and in preparation for spaceflight experiments in the International Space Station, in order to fulfill these objectives.

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