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Mechanotransduction as an Adaptation to Gravity

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Gravity has played a critical role in the development of terrestrial life. A key event in evolution has been the development of mechanisms to sense and transduce gravitational force into biological signals. The objective of this manuscript is to review how living organisms on Earth use mechanotransduction as an adaptation to gravity. Certain cells have evolved specialized structures, such as otoliths in hair cells of the inner ear and statoliths in plants, to respond directly to the force of gravity. By conducting studies in the reduced gravity of spaceflight (microgravity) or simulating microgravity in the laboratory, we have gained insights into how gravity might have changed life on Earth. We review how microgravity affects prokaryotic and eukaryotic cells at the cellular and molecular levels. Genomic studies in yeast have identified changes in genes involved in budding, cell polarity, and cell separation regulated by Ras, PI3K, and TOR signaling pathways. Moreover, transcriptomic analysis of late pregnant rats have revealed that microgravity affects genes that regulate circadian clocks, activate mechanotransduction pathways, and induce changes in immune response, metabolism, and cells proliferation. Importantly, these studies identified genes that modify chromatin structure and methylation, suggesting that long-term adaptation to gravity may be mediated by epigenetic modifications. Given that gravity represents a modification in mechanical stresses encounter by the cells, the tensegrity model of cytoskeletal architecture provides an excellent paradigm to explain how changes in the balance of forces, which are transmitted across transmembrane receptors and cytoskeleton, can influence intracellular signaling pathways and gene expression.

Keywords: gravity, earth (planet), mechanotranduction, cytoskeleton, tensegrity, microgravity, genomics, epigenetic

INTRODUCTION

Life evolved from the sea. Organisms borne in the primitive sea about 30 million years ago had evolved in water without a large influence of gravity on earth. Underwater, gravity is almost compensated by buoyancy, "the upward force exerted by a fluid that opposes the weight of an immersed object." In contrast, other forces such as drag and lift became significant in an aquatic environment. These flow forces vary in magnitude and direction, and under specific conditions, can even reach higher values than gravity. In addition, viscous forces and the lack of surface tension under immersed conditions are also important in the aquatic environment (1). Some aquatic species may still sense gravity. However, the pressure around them and the internal organs are able to counterbalance the force of gravity (2). For example, the swim bladder, the evolutionarily homologous to the lungs, is

an internal gas-filled organ of many bony fish. It has flexible walls that contract or expand according to the ambient pressure to obtain neutral buoyancy and ascend and descend to a large range of depths. However, some aquatic species appear to use gravity as a directional cue. Throughout evolution, aquatic species have adapted to their environment. In addition to inner ear hair cells, aquatic vertebrates have hair cells on the surface of their body in the lateral line system. These mechanoreceptors provide a highly sensitive and versatile hydrodynamic sense that is used in a wide range of behavior (3). In order to understand how these receptors mechanically filter signals, a theoretical model of the superficial neuromast in the fish lateral line system has been developed (4). Other species such as crustaceans contain a great variety of sensilla along their antennules that enable them to sense both hydrodynamic and chemical stimuli in aquatic environments (3).

In spite of the more or less constant gravitational force on Earth, mechanical load on organisms on land is approximately 1,000 times larger than in water. About 4 million years ago, the first terrestrial organisms, plants appeared on the land from the sea. The terrestrial plants have adapted to and evolved on the land environment, so that they can extend their roots downward in soil and their shoots upward against $1 \times g$. Land species began to develop adaptive mechanisms to orient themselves to the gravity vector.

An attractive hypothesis that gravity might have played a key role in evolution comes from studies in snakes (5). On Earth, snakes live in different environments. For example, tree snakes crawl up trees and have to cope with gravity when compared to land snakes that spend most of their life in a horizontal position or even the neutrally buoyant sea snakes. Lillywhite found out that the heart of the tree snake was closer to the brain when compared to the other snakes. These studies indicate that even without altering the magnitude of gravity, changes in orientation with respect to the direction of a gravitational force may have played an important role in the evolution of the species on Earth. In this specific example, gravity may have determined the location of the heart and perhaps other internal organs in snakes.

What about species that "returned to the water" as descendants from non-aquatic ancestors, like cetaceans? The origin of whales is one of the best examples of macro-evolutionary change in vertebrates. As they became obligated marine, they had to adapt to the new environment. Studies from fossil suggest that this adaptation took less than 15 million years and different organs followed distinct evolutionary patterns. Although the anatomy and function of the sensory organ differ among them, all take advantage of the non-compressive and dense nature of the water and fossil anatomic analysis of early cetaceans showing parts of the snout of early cetaceans show extreme pitting and grooving. These findings suggest that the tip of the snout was important as mechanoreceptor. In addition, during aquatic adaptation, some organs involved in olfaction, vomeronasal sense, and balance underwent an involution process (6). Another example of evolution in cetaceans is the hearing sense. The ear of whales, initially intended for hearing on the surface, became adapted for hearing underwater and was substituted by the mandible and the mandibular fat pad. "Pakicetids, the earliest archaeocetes, had a land mammal ear for hearing in air, and used bone conduction underwater. Remingtonocetids and protocetids were the first to display a genuine underwater ear where sound reached the inner ear through the mandibular fat pad. Basilosaurids and dorudontids showed further aquatic adaptations of the ossicular chain and the acoustic isolation of the ear complex from the skull" (7, 8).

Life on Earth has developed in the presence of gravity. Therefore, it is important to understand the effect of this force on the evolution of terrestrial life. "Although it is clear that physical forces, such as those due to gravity, are fundamental regulators of tissue development, little is known about how living cells sense these signals and convert them into a biochemical response. This transduction process is known as mechanotransduction" (9). Certain cells have evolved specialized structures, such as statoliths in plants and otoliths in hair cells of the inner ear, to respond directly to the force of gravity.

Gravity alterations experienced by astronauts have important effects on the human body. For example, when standing upright on Earth, fluids are accumulated in the lower extremities. In contrast, in microgravity, fluids are displaced to the thorax and head. As a result, there is an increase in cardiac filling and diuresis, and the overall effect is a reduced blood volume. In addition, microgravity reduces the loading of skeletal muscles leading to decreased muscle mass, muscle strength, and endurance. Moreover, low gravity increases loss of calcium from the bone and has an inhibitory effect on bone formation and subsequent vulnerability to fracture. On entering microgravity, removal of gravitational information results in altered body and spatial orientation and visuo-motor coordination (10).

Microgravity has also a significant impact on the physical forces encountered by cells. By performing studies in a reduced gravity environment, we may get better understanding on how gravity affected life on Earth. However, performing experiments in the space are difficult for obvious reasons. Alternatively, several ground-based models have been designed, including random positioning machine, diamagnetic levitation, and hind limb unloading. In addition, some cell culture systems, such as the clinostat and rotary cell culture system, also simulate microgravity (11).

In vitro studies have demonstrated that cell proliferation, growth, differentiation, signaling, shape, and gene expression are all altered by microgravity (12–14). However, the mechanisms responsible for this adaptation to changes in gravity remain controversial. This information is important not only for the effects of spaceflight at the tissue/organism level but also to study the response of cells to the decrease of force under microgravity (15).

In this manuscript, we will review first how individual cells (both prokaryotic and eukaryotic) are able to sense gravity; then, we will discuss how more complex eukaryotic tissues (plants, animals, developmental windows for programing) and organisms respond. Finally, the molecular mechanisms that are implicated in cytoskeletal signal transduction and the concept of tensegrity will be discussed.

Bacteria

Previous investigations have shown that spaceflight increase the risk of infections in both humans and animals (16, 17). Experiments with various microbes have found that microgravity

has a significant impact on several factors, such as growth, morphology, metabolism, genetic transfer, and viral reactivation (18). For example, it has been shown that bacterial growth increases by 88% in microgravity when compared to ground controls (19). Interestingly, they also observed an increased resistance of *S. aureus* and *Escherichia coli* to antibiotics (20). Simulated microgravity using the RWV has also observed an enhanced virulence of a Gram-negative bacterial pathogen, *Salmonella* Typhimurium (21). Typhimurium showed increased resistance to acid, to osmotic and thermal stresses, and an increased capacity to survive inside the macrophages, when compared to cells grown under normal gravity conditions (21–23). In conclusion, the ability of bacteria to proliferate and grow under low gravity conditions is enhanced. Therefore, there is an increased susceptibility to infections in microgravity (22).

In addition, microgravity seems to significantly affect gene expression in prokaryotes (24). For example, DNA microarrays were used to investigate changes in gene expression of *Salmonella* Typhimurium exposed to simulated microgravity. Compared to normal gravity conditions, simulated microgravity differentially regulated the expression of 163 genes encoding transcriptional factors, virulence factors, lipopolysaccharide biosynthetic enzymes, ribosomal proteins, iron utilization enzymes, etc. (23). Thus, microgravity is an important environmental factor able to reprogram gene expression (24).

How Do Microbes Respond to Microgravity?

The response of a microbial cell to changes in extracellular solute concentration is critical for cellular function and survival. Microbes sense changes in osmotic pressure gradients *via* tension in the cell membrane (25, 26). Several studies have demonstrated that cells recognize changes in gravity at the cell surface (27, 28). Indeed, microgravity causes changes in the cell surface of microbial cells (23, 26–29). It has been shown that changes in fluid shear force can affect bacterial adhesion (30). Therefore, it appears that decreased distortion in the cell surface, caused by lower level of fluid shear forces, is an important mechanism for cellular mechanotransduction under microgravity conditions. Studies performed in *E. coli* FimH provide a model for mechanotransduction in microbes (27, 31). In this model, a sensor protein embedded in the prokaryotic cell membrane has two domains connected by a flexible linker region. One domain is embedded in the membrane and can serve to initiate signaling inside the cell. The other domain is extracellular and can initiate conformational changes in the linker in response to the environment.

Yeast

Previous studies have examined the effect of microgravity on cell function, morphology, and genomic expression of the yeast *Saccharomyces cerevisiae*. With the exception of a shortened lag phase, yeast cells grown under microgravity conditions did not vary from the controls. However, they differed in the formation of polarity, as shown by abnormal budding compared to the bipolar pattern present in the controls. The aberrant budding was accompanied by an increased tendency of cells to clump (32).

Additional investigations using DNA microarray (33) found that 1,372 genes (36%) were significantly altered by exposure to low-shear modeled microgravity, from which 26% of them were environmental stress responses genes. Notably, one of the genes most dramatically affected was HSP30 which is known to be responsive to high hydrostatic pressure (34, 35). In addition, they also found significant changes in the expression of genes associated with the establishment of polarity, bipolar budding, and cell separation. Thus, low-shear environments may significantly alter yeast gene expression and phenotype, as well as evolutionary conserved cellular functions such as polarization.

Cell polarity refers to "spatial differences in the shape, structure, and function of cells." Budding is a "form of asexual reproduction in which a new organism develops from an outgrowth or bud due to cell division at one particular site." Normal yeast cells assemble buds in response to a well-orchestrated program that promotes polarized growth. Investigations performed in yeast exposed to simulated microgravity show remarkable differences in the establishment of cell polarity, bipolar budding pattern, and cell separation (32, 33). Specifically, they identified four genes involved in bud pattern selection (*BUD5*, *RAX1*, *RAX2*, and *BUD25*) and three genes related to cell separation (*DSE1*, *DSE2*, and *EGT2*). *BUD5* regulates the activity of the small GTPase Cdc42 and plays a key role in the initiation and establishment of cell polarity during budding (36). In addition to a random budding phenotype, cells grown in microgravity were also found in clumps containing five or more cells (32). *DSE1*, *DSE2*, and *EGT2* are part of a cell division program (37) and rely on the accuracy of the cell polarization process (38, 39). Thus, "the low microgravity-induced defect in cell polarity could be the underlying mechanism for both the random bud scarring and the formation of the large cellular aggregates observed" (32).

Yeast cells must maintain cell wall integrity when exposed to changes in the extracellular environment such as osmotic stress and oxidative stress (40). Moreover, during polarized cell growth, yeast cells have to preserve cell shape to avoid rupture during bud formation and subsequent cell division. The MAPK signaling pathway is primarily responsible to regulate changes to the cell wall under these conditions. Microarray analysis identified two protein phosphatases SDP1 and PTP2 upregulated by microgravity. Both proteins inhibit the MAPK signaling pathway. Therefore, downregulation of the MAP kinase by SD1 and PTP2 can compromise cell shape and polarity. "The changes in phenotype, together with the microarray results, implicate the cell wall integrity MAP kinase pathway as a possible mechanism for sensing the low shear microgravity environment" (33).

Signaling Pathways Regulating Chemotaxis

Chemotaxis is defined as the movement of a motile cell or organism, or part of one, in a direction corresponding to a gradient of increasing or decreasing concentration of a particular substance. Directed cell migration and cell polarity are basic migratory systems that have been conserved throughout evolution (41–43). Cells have evolutionarily developed a mechanism to respond to an extracellular gradient of chemoattractant *via* the PI3K

signaling pathway. The PI3K family proteins are lipid kinases that phosphorylate phosphatidylinositol (PI) or phosphatidylinositol phosphate. PI3K is primarily responsible for the production of $PI(3,4,5)P_3$ at the leading edge of the plasma membrane in response to extracellular stimulation (44).

Several studies in *Dictyostelium* have demonstrated that Ras regulates both directional movement and cell polarity (45, 46), but independently from each other, as shown in one of the RasGEF mutants Aimless, where *aimless* null cells show a reduced directionality without affecting cell polarity or chemotaxis speed (47, 48). Ras is quickly activated after stimulation in *Dictyostelium* cells. Activated Ras is limited to the leading edge of the cell. Ras stimulates PI3K, which triggers an intracellular $PI(3,4,5)P_3$ gradient at the leading edge and F-actin polymerization (44).

Besides PI3K, cells have PI3K-independent pathways for F-actin polymerization and polarization. The TOR pathway was found to control cell polarity *via* Ras (49). TOR is a PI3K-related kinase with two protein complexes, TORC1 and TORC2. One of the principal functions of TORC2 is actin cytoskeleton rearrangement. The mechanisms by which TORC2 regulates actin reorganization is not well defined; although some studies have implicated Slm1 and Slm2, members of a family protein bind to $PI(4,5)P_2$ and TORC2. Slm1 and Slm2 are activated by TORC2, and inactivation of Slm1 and Slm2 diminishes polarized cortical actin distribution (50, 51). These investigations from *Dictyostelium* demonstrate an important role of TOR complexes in chemotaxis *via* Ras. Therefore, Ras may regulate chemotaxis through PI3K and TOR pathways.

In summary, a model for feedback loop-mediated directional sensing and cell polarization has been proposed. "The chemoattractant locally stimulates Ras at the presumptive leading edge (site of the membrane closest to the chemoattractant source) where Ras locally activates PI3K. There is a local polymerization of F-actin at the presumptive leading edge which is dependent on Ras/PI3K and Ras/TORC2 pathways. Locally produced $PI(3,4,5)P_3$ and F-actin-mediated Rac activation induces further F-actin polymerization and chemotactic movement of the cell" (44).

PLANTS

Mechanotransduction in Plants

The direction of growth of plants is primarily regulated by gravity (52). Plants can re-orientate themselves with respect to gravity. When a plant is turned in the gravitational field, the change in orientation is perceived by its organs. These organs respond to this stimulus by bending their extremity to recover the normal orientation with respect to gravity (53).

Movement of Amyloplasts in Response to Gravity

How do plants sense the direction of gravity? Two hypotheses have been proposed: the gravitational pressure model (54) and the starch-statolith hypothesis (55). The latter has been strongly supported by a variety of experimental approaches in several plants. Amyloplasts are defined as "non-pigmented organelles found in some plant cells. They are responsible for the synthesis and storage of starch granules, through the polymerization of glucose." In most cases, statoliths are starch-accumulating amyloplasts that can sediment in the direction of gravity within specialized cells (statocytes) present in the columella and shoot endodermal cells. Columella endodermal cells have the endoplasmic reticulum (ER) on the peripheral side of the cells. A specialized form of ER, called nodal ER, is restricted to a peripheral layer underneath the plasma membrane (56). It has been suggested that the nodal ER could provide directional cues by identifying the orientation of the root in relation to gravity. In contrast, shoot endodermal cells are occupied by a large central vacuole. Genetic studies of *sg r* mutants have found that the contribution of vacuoles to gravity is by modifying amyloplast sedimentation (57). Therefore, columella and shoot endodermal cells seem to play key roles for gravity sensing on shoots and roots.

Past investigations have demonstrated an association between amyloplast movement and response to gravity (58, 59). Sack and Leopold (60) have described amyloplast movement in living statocytes. They observed that amyloplast sedimentation happened within the minimum time of continuous stimulus necessary to trigger gravity perception. Morita's group (61) suggested that movement of amyloplasts toward the gravity vector is crucial to trigger gravity perception.

Cell Signaling Mechanisms after Amyloplast Sedimentation

Cytosolic ions mediate gravity signaling. Calcium is the most abundant ionic second messenger in plants. However, changes in cytoplasmic pH are also important regulators of cell function. It has been demonstrated, for example, that cytosolic calcium is transiently activated by gravity (62). They observed that after gravistimulation, there was an initial cytosolic calcium spike for 20–25 s, followed by a much longer shoulder that peaked around 90 s after the change in orientation of the plant. The investigators suggested that the "spike could be related to the early steps of gravisensing, whereas the shoulder could be related to the movement of the amyloplasts."

Besides the ionic signaling, several proteins have been identified to participate in the cell signaling pathways related to gravitropism. The ARG1/RHG protein, for example, is mostly associated with cell membrane but a small portion also appears to be associated with the actin cytoskeleton (63). Functional ARG1/RHG protein is required for alkalinization in the root columella cells following gravistimulation. ARG1/RHG is present on several endomembrane systems, such as ER, the Golgi apparatus, and endosomes (64). Therefore, ARG1/RHG protein might function in the vesicular trafficking required for the alkalinization of columella cells.

Gravity Sensing and the Cytoskeleton

The cytoskeleton was the best candidate for transmitting the force exerted by gravisensors to the mechanoreceptors. Actin filaments in particular have been thought to play a major role in gravity sensing. It has been shown that in microgravity, the statoliths are not distributed at random in the root statocytes. When lentil seedlings are grown in a $1 \times g$ centrifuge in space and then placed under microgravity conditions for various periods of time, the

amyloplasts move in the direction of the proximal wall (65, 66). This movement is attributed to the presence of myosin around the amyloplasts (67). Therefore, the movements of the amyloplasts detected in microgravity demonstrate the interactions between amyloplasts and cytoskeleton. The role of cytoskeleton has been also demonstrated using the *arg1* mutant of *Arabidopsis* (63), which showed an altered response to gravity. However, other studies have shown conflicting results (68–70). Interestingly, organ curvature exceeding 90° was found in plants exposed to latrunculin-B to disrupt actin organization and in dominant actin mutant (69, 70), suggesting that actin cytoskeleton may be important to fine-tune the gravitropic response.

In summary, "gravisensing cells (statocytes) contain movable amyloplasts whose potential energy is apparently used to activate calcium channels by exerting tension on the actin network and/or pressure on the cytoskeleton elements lining the plasma membrane. The chain of events that follows remains to be further elucidated but includes pH changes in the cytosol and cell wall" (62). Transduction ends with relocation of the auxin, a signaling protein that regulates several plant functions, including cell elongation, which reorients the root tip in the direction of gravity (53).

ANIMALS

Vestibular System
Mechanotransduction by Hair Cells
The vestibular system is important to maintain the body equilibrium under gravity. The detection of the gravitational force requires specific receptors. This is achieved by two otolithic organs of the vestibular system, the utricle and saccule (71). These gravity receptors utilize a layer of calcium carbonate (otoconia) lying over the sensory receptor areas. The shearing force produced by the otoconia displaced against the stereocilia of the sensory hair cells allows the detection of linear accelerations, and gravity (72).

"Hair bundles are the mechanosensitive organelles that transduce vestibular stimuli into electrical signals" (73). "Each vestibular end organ contains thousands of hair cells. Each hair cell has one bundle that contains 30–300 microvilli, or stereocilia. Stereocilia are composed of cores of hundreds of actin filaments covered by an extension of the cell membrane" (74). The actin filaments taper near their base and are anchored to the cell body in a thickened region known as the cuticular plate (75). This arrangement allows the stereocilia to hinge around the insertion into the cuticular plate (76). Furthermore, stereocilia are connected together, within a hair bundle and not to neighboring bundles (77). Therefore, stimulation of the hair bundle does not induce separation of the stereocilia but rather brings uniform bundle deflections (78). "The stereocilia are arranged in a staircase-like array with a single true cilium, the kinocilium, located adjacent to the top of the staircase. The kinocilium does not exhibit mechanosensitivity but provides a connection of the apex of the hair bundle to overlying structures and it may also serve to organize proper formation of the bundle during development" (79).

Transduction channels are located at stereociliary tips (80). Mechanotransduction is initiated when mechanical stimuli activate the stereocilia at the apical surface of the hair cells. Movement of the stereocilia induces the opening of cation channels with subsequent activation, changes in the membrane potential of the hair cells and release of neurotransmitters to send the information to the brain (81). The molecules that form the transduction channel have not been identified. However, recent studies in mice (82) have identified key roles for transmembrane channel-like 1 and -like 1 in hair cell mechanotransduction. The mechanisms by which tip links open the transduction channels are not know. However, two models for channel coupling and gating have been proposed, The Tethered-Channel Model and The Lateral Tension Model. In the first one, "the channel is connected to the cytoskeleton and to the tip link, so that forces in the tip link are propagated *via* protein-protein interactions to the channel." In the lateral tension model, "the channel responds to tension in the stereocilia tip membrane. Increased tension in the tip link would then increase membrane tension and open channels" (83).

Vestibular Response to Microgravity
The maturation of gravity sensing is genetically programed and also depends on the exposure to the gravity (84, 85). The nervous system probably needs environmental experience to calibrate the gravity information during critical periods of development and several studies have shown the sensitivity of the organisms to the alteration of gravity during their development (86–90). The peripheral sensory organ adapts to the level of gravity by adjusting the mass of otoconia and the innervation of sensory epithelium. Understimulation, such as caused by microgravity, can delay the maturation of neural connections during the formation of the vestibular apparatus. "All together, these results provide further evidence that the gravistatic sensory system has a genetically controlled phase of development and a stimulus-controlled phase for fine-tuning synaptic terminals. Therefore, the level of gravity plays a critical role in fine-tuning of axons and is required for appropriate development of the projections from graviceptors to the brain and spinal cord" (72).

Bone
Role of Osteocytes and Canaliculi in Bone Mechanotransduction
The skeleton has the capability to remodel in response to mechanical stimuli (91). Osteocytes comprise over 90% of all bone cells in adults. Osteocytes are considered the terminal differentiation stage of osteoblasts and are distributed all through the mineralized matrix, in particular the cortical bone. These cells are connected to each other and to cells on the surface *via* dendritic processes present inside small canals called canaliculi (92). Because of their distribution and extensive network connections, osteocytes are considered to play a central role in mechanotransduction of bone cells by sensing mechanical signals and regulating bone resorption and formation (93). Using live imaging of osteocytes expressing green fluorescent protein, Dallas and colleagues (94) demonstrated that osteocyte embedded in bone can extend and retract their dendritic processes and undergo cell body deformation. In addition, it has been suggested that osteocytes are activated by strain-driven motion of interstitial fluid through the

lacunocanalicular porosity (95–97). *In vitro* studies have demonstrated that the flow of fluids through the canaliculi stimulates the release secondary messengers by osteocytes, including ATP, nitric oxide, Ca^{2+}, and prostaglandins (98–101). These studies suggest that the movements of the fluids through the channels could be one of the main mechanical stimuli on osteocytes (11). The Wnt/β-catenin signaling pathway is an important modulator of bone mass and bone cell functions. This pathway is critical for osteoblasts differentiation, proliferation, and synthesis of bone matrix. The Wnt/β-catenin pathway seems to be important in osteocytes to transfer mechanical signals to cells located on the bone surface (102).

Effects of Microgravity on Bone Cells

Under microgravity conditions, bones no longer have to fight against Earth's gravitational force during locomotion and less mechanical strain is applied to the skeletal system. Reduced bone strain is widely accepted to cause progressive bone loss under microgravity (103). There are four major types of bone cells: mesenchymal stem cells (MSCs), osteoblasts, osteocytes, and osteoclasts.

Mesenchymal stem cells are multipotent cells with high replication capacity and the potential to differentiate into different lineages of mesenchymal tissues, including bone, cartilage, fat, muscle, and marrow stroma (104). Mechanical forces can enhance the osteogenic differentiation of MSCs, while a lack of mechanical stimuli can induce adipogenesis (105). Past studies have demonstrated that simulated microgravity inhibits the osteogenic differentiation of MSCs and osteogenic precursor cells (106). Some authors attribute these changes to F-actin cytoskeletal disturbances and alterations of Rho GTPase activity (107).

Osteoblasts are derived from MSCs. Osteoblasts control bone formation and participate in the regulation of bone homeostasis (108). Microgravity inhibits the differentiation of osteoprogenitor cells into mature osteoblasts (107). Osteoblasts are considered responsible for the bone loss induced by microgravity, given their decreased proliferation, reduced differentiation, and decreased response to local factors under low gravity conditions. Previous investigations have shown that microgravity disrupts osteoblast microfilaments, resulting in defective bone formation (109, 110). In addition, low gravity can promote apoptosis by altering focal adhesions (111).

Osteocytes have been implicated as key effectors of microgravity-induced bone loss (112). Osteocyte apoptosis was found after a 2-week flight accompanied by an increasing the number of functionally active osteoclasts (113). Hypogravity compromises the cytoskeletal architecture and suppresses the gap junctions of osteocytes. Given the key role of gap junctions in cell-to-cell communication, these changes are going to compromise overall cell function (114).

Osteoclasts are multinucleated bone-resorbing cells (115). Osteoclast differentiation is enhanced in microgravity (116). Microarray analysis of osteoclasts in the modified rotating wall vessel RCCS showed an upregulation of genes involved in osteoclast differentiation (117). Several studies indicate that bone loss under microgravity is caused by a decrease in osteoblast function and an increase in osteoclastic activity (118, 119).

Lung
The Lung in Gravity

The normal lung is markedly influenced by the presence of gravity. To better understand the effects of microgravity on the lung, it is important first to describe how gravity affects perfusion and ventilation in dependent versus non-dependent regions of the lung.

The zone model of pulmonary perfusion establishes that regional perfusion depends on the balance between pulmonary arterial pressure, pulmonary venous pressure, and alveolar pressure (120). Given the low perfusion pressures in the pulmonary circulation, hydrostatic pressure caused by gravity is important to determine pulmonary perfusion. Thus, in the lower parts of the lung, blood flow depends on difference between arterial and venous pressure. In contrast, at the top of the lung, and given the decrease of hydrostatic pressure by gravity, pulmonary pressures can fall below alveolar pressure and compromise blood flow. Therefore, there is a vertical gradient in blood flow in the different regions of the lung with blood flow greater in the dependent portions of the lungs.

The Slinky model of pulmonary ventilation (121) determines that the behavior of a spring is similar to the lung. If the spring is stretched, the loops at the top are more distant from each other than at the bottom. This is similar to alveolar size under resting conditions, with alveoli at the top being bigger than at the bottom. However, if the string is stretched further (to simulate inspiration), and due to the elastic recoil forces of the spring, the loops in the lower part of the spring are more distant apart from each other than in the upper part (and by comparison, ventilation is greater in the lower parts of the lung). If the effects of gravity are removed, this model would predict uniform alveolar size, ventilation and perfusion (122).

In summary, "gravity causes uneven ventilation in the lung through the deformation of lung tissue (Slinky effect), and uneven perfusion through a combination of the Slinky effect and the zone model of pulmonary perfusion" (122).

The Lung in Microgravity
Lung Volumes and Expiratory Flows

Vital capacity showed an initial small reduction, but by day 4 in microgravity it returned to pre-flight values. In contrast, functional residual capacity fell in microgravity. This was probably caused by the elimination of the "push-down" effect of the abdominal contents. Unexpectedly, residual volume decreased in microgravity when compared to normal gravity. The likely explanation is that "under gravity, dependent regions of the lung reach their local residual volume before the entire lung does, and gas remains trapped in these regions. However, in microgravity, the uniform alveolar expansion permits a more uniform overall emptying of the lung and a lower total residual volume" (123).

Ventilation

Based on the Slinky model, one would expect that pulmonary ventilation in all regions of the lung should be identical under low gravity conditions. However, when single-breath tests

were performed in spaceflight (124), ventilatory heterogeneity persisted to some degree. This could be perhaps explained by differences in regional lung shape. Another indicator of regional differences in ventilation is the presence of cardiogenic oscillations. Cardiogenic oscillations are generated by the expansion of the heart during diastole on the nearby lung, implying differences in ventilation between the lung close and distant to the heart. Contrary to expected, cardiogenic oscillations persisted near to 50% of the results under gravity. Therefore, these findings and the previous studies from the single-breath washout experiments demonstrate the presence of ventilatory heterogeneity in microgravity (125). To support these observations, other studies have also found that the lack of ventilatory uniformity during tidal breathing in the upright position was not primarily gravitational (126). Therefore, it looks like that the elastic properties of the lung have greater impact than gravity during tidal breathing.

Blood Flow

The effects of gravity on uneven distribution of pulmonary perfusion were tested under microgravity conditions using the hyperventilation-breathhold single-breath measurements. Under low gravity, the indicators of uneven pulmonary perfusion such as the size of cardiogenic oscillations in expired CO_2 and the height of phase IV, were significantly reduced. The terminal change in expired CO_2 was also reduced in microgravity, indicating more uniform blood flow between lung units that close and those that remain open at the end of expiration. A possible explanation of this observation is the disappearance of gravity-dependent distribution of blood flow. However, the presence of residual cardiogenic oscillations in expired CO_2 implies a persisting non-homogenous distribution of perfusion even in the absence of gravity, probably in lung regions that are not within the same acinus (127).

Gas Exchange and Ventilation–Perfusion Matching

The principal change observed in microgravity was a slight decrease of alveolar ventilation (128). There was also a significant drop in resting tidal volume and simultaneous increase in respiratory rate (129). This was associated with a reduction in the physiological dead space. Given that the degree of heterogeneity of both ventilation and perfusion in the lung were reduced in microgravity, one would expect the V/Q to be reduced as well. However, the V/Q did not change when compared to normal gravity (130). The explanation is that under $1 \times g$, the areas of high ventilation had also high perfusion and vice versa. Therefore, "gravity imposes common effects on both ventilation and perfusion (the zone and Slinky models) serving to maintain a high gas exchange efficiency in the lung" (122).

Conclusion

The zone model of pulmonary blood flow and the Slinky model of lung deformation together provide a basis to understand how gravity affects lung function and how the lung changes in the absence of gravity. Under gravity, and despite the regional differences in ventilation and perfusion, the V/Q ratio remains constant and provides efficient gas exchange. In a weightless environment, and even with more uniform distribution of both ventilation and perfusion, the gas exchange seems to be no more efficient than in gravity. In addition, and contrary to other organs, the lung does not experience significant structural changes when gravity is removed and continues to function well [for a more comprehensive review, please see the excellent article by Prisk (122)].

Lung Gravisensors

Lung development is well known to be influenced by a highly active mechanical environment (131, 132). One possible interpretation of the link between physical force and development is the presence of gravisensors that have allowed organisms to adapt to gravity during evolution (133) and development (134). Previous studies have shown that lung cytoarchitecture is exquisitely sensitive to fluid distension in the womb, a process that would allow for sensing of gravitational forces on the developing conceptus (135, 136).

Parathyroid hormone-related protein (PTHrP) has been shown to be essential for the development and homeostatic regulation of lung and bone (137). PTHrP is expressed in a wide variety of tissues, including the uterus (138, 139), bladder (140), lung (141), and bone (142). During gestation, PTHrP levels increase in the distending uterus and then rapidly decrease when the uterus is evacuated at birth, suggesting that mechanical stretch causes the increased PTHrP expression. Moreover, studies by Torday's research group have demonstrated that expression of PTHrP by the pulmonary epithelium is sensitive to stretch (136) and that production of PTHrP is necessary for the normal alveolar development (143).

Interestingly, prior investigations have demonstrated that PTHrP is a gravisensor in both lung and bone (144, 145). When either rat lung type II epithelial cells or human UMR 106 bone cells were suspended in a rotating wall vessel bioreactor, to simulate microgravity, they lost their PTHrP receptor-mediated signaling mechanism within 6–8 h (144). As a result, the cells established a new, lowered baseline of PTHrP expression. If the cells were put back into gravity, PTHrP signaling was restored within 24 h, suggesting that this was a reversible process. They concluded that PTHrP signaling pathway constitutes a mechanism by which these cells can sense gravity and alter their cellular and biochemical environment accordingly. In further support of the relevance of this gravisensing mechanism to microgravity adaptation, Torday's group analyzed bones from rats flown in space on NASA Mission SL-2 (144, 145) Analysis of PTHrP receptor expression by the femurs and tibias of these animals revealed that PTHrP expression was 60% lower than in the bones from control ground-based rats. Interestingly, there were no differences in PTHrP expression by parietal bone from space-exposed versus ground-based animals, indicating that the effect of microgravity on PTHrP expression is specifically the result of the unweighting of the weight-bearing bones.

In conclusion, PTHrP is a mechanosensor found to be sensitive to changes in gravity environment. The link among physical force, development, and homeostasis support the hypothesis that there are gravisensors that have allowed organisms to physiologically adapt to gravity (137).

Gene Expression Response to Microgravity

Several large-scale genome-wide studies have been performed in a variety of organisms such as human, rat, mouse, *Xenopus*, yeast, *Caenorhabditis elegans*, and *Drosophila* to understand the impact of gravity on living organisms (146).

Cells

Several studies have investigated the impact of low gravity on gene expression in isolated cells. Wang's group (147) examined the effects of microgravity on WI-38 human fibroblasts. Their data show that spaceflight activates a group of genes involved in oxidative stress, DNA repair, and fatty acid oxidation. Clement et al. (148) investigated the effects of simulated microgravity on a keratinocyte cell line. They found that exposure to microgravity for a short period of time (3–4 days) had no persistent effect on gene expression. In contrast, longer exposure (9 or 10 days) exhibited substantial alterations in gene expression. Their results suggest that longer exposure to microgravity tends to have lasting effects on gene expression. Their experiments are also in agreement by studies performed by Lu et al. (149) demonstrating mutations in the content of albumin, globulin, and prolamine in rice seeds that were flown on a recoverable satellite for 15 days. These changes were stably inherited from several generations on earth. Altogether, these studies demonstrate that exposure to microgravity could have a long-lasting impact on gene expression at the cellular level.

Mammalians

Recent studies have investigated the impact of spaceflight on mammary transcriptome of late pregnant rats (150). Microarray analysis of the dams revealed that "alterations in gravity affected the expression of genes that regulate circadian clocks and activate mechanotransduction pathways. Changes in these systems may explain global gene expression changes in immune response, metabolism, and cell proliferation" (150).

The circadian system is important to synchronize the physiological oscillations with the external environment signals such as light, exercise, stress, and gravity. Several studies have demonstrated that changes in gravity significantly affect circadian rhythms of behavior, hormones, body temperature, and metabolism (151–156). Specifically for gravity, the vestibular system is the primary sensor that detects changes in gravitational force; impulses from these receptors are sent to the central circadian clock in the suprachiasmatic nuclei. When circadian rhythms are disrupted, an organism's ability to respond appropriately to a physiological stressor is inhibited. Transcriptional signatures of microgravity rats showed changes in expression of multiple core clock and clock regulatory genes, indicating that microgravity affect the expression of genes that regulate circadian clocks (150).

Impact of Microgravity on Epigenetic Modifications

Epigenetics has been defined as the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence. At least three systems including DNA methylation, histone modification and non-coding RNA-associated gene silencing are currently considered to initiate and sustain epigenetic modifications. Epigenetic changes, in particular DNA methylation, occur in response to various environmental factors. Epigenetic changes are inherited in somatic cells and provide a potential mechanism by which environmental effects on the epigenome can have long-term effects on gene expression (157). Epigenetic modifications are regulated by a family of enzymes, called DNA methyltransferases, transferring a methyl group to DNA. Expression of DNA methyltransferase 3α was decreased in microgravity when compared to $1 \times g$ controls (150). DNMT3A is referred to as a *de novo* methyltransferase. DNMT3a can also mediate methylation-independent gene repression.

They also observed upregulation of Kruppel-like factor 4 (KLF4) in microgravity of mammary tissue. KLF4 is a member of the KLF family of transcription factors and regulates proliferation, differentiation, and tissue development, in part *via* chromatin modification. KLF4 has been found to participate in the reprograming of somatic cells into pluripotent stem cells *via* downregulation of DNA methylation on certain genes (158).

Members of the forkhead/winged helix transcription factor family were also affected by alterations in gravity in these investigations. FOXN3 was upregulated in mammary of spaceflight-exposed rats. FOX proteins participate in regulation of gene expression involved in cell growth, proliferation, differentiation, and longevity. They are able to control DNA methylation by binding condensed chromatin and recruiting other transcription factors and histone modification enzymes.

Other investigations, using simulated microgravity, have also observed epigenetic changes in human lymphoblastoid and lymphocyte cells (159, 160). However, none of these studies have demonstrated that these changes are heritable. Therefore, it is possible that microgravity only affects the transcription factors that are possibly linked with epigenetic effects on chromatin structure and cellular cytoskeletal physiology. In contrast, spaceflight induced both transient and heritable alterations in DNA methylation and gene expression in rice (161).

In summary, these studies show changes in genes that modify chromatin structure and methylation, suggesting that long-term adaptation to gravity might be mediated by epigenetic changes in DNA.

CYTOSKELETON

Role of Cytoskeleton as Gravity Sensor

Mechanical forces, such as those due to gravity, play an important role in tissue development. However, the mechanisms by which living cells sense these mechanical signals and convert them into an intracellular response are still not well characterized. It is also controversial whether the forces induced by gravitational field on the cells are too small to induce any response (15). Despite this theoretical consideration, there are accumulative experimental data demonstrating that cultured cells are sensitive to gravity (12–14). Moreover, there is clear evidence that certain cells, such as the statoliths in plants and the otoliths of the inner ear, have developed specialized structure in response to gravity, indicating their ability to sense these mechanical signals.

The cytoskeleton provides shape and mechanical strength to cells. It consists of actin, microtubules, and intermediate filaments (162). The cytoskeleton is able to sense changes induced by gravity. However, to do so and promote changes at the cellular levels, this force has to be transmitted first to receptors on the cell membrane sensitive to mechanical signals and then to downstream signaling pathways that eventually will affect cell function.

Tensegrity Model of Mechanotransduction

Past studies have suggested that "cells may sense mechanical stresses, including those due to gravity, through changes in the balance of forces that are transmitted across transmembrane adhesion receptors that link the cytoskeleton to the extracellular matrix and to other cells" (163–165). The mechanism by which these mechanical signals are converted into an intracellular response could be explained by a model, known as *tensegrity*, that describes how cell architecture detects and responds to stimuli (166).

In this model, the cellular response to stress differs depending on the level of pre-stress (pre-existing tension) in the cytoskeleton, and it involves all three cytoskeletal components as well as nuclear scaffolds (164, 165, 167, 168). The tensegrity model suggests that "cellular control lies in the balance of forces that are transmitted across cell surface adhesion receptors, through the cytoskeleton, and into the nucleus" (169). In this sense, we can think of integrins and other transmembrane adhesion receptors as mechanoreceptors. Past investigations have shown that alterations in the cellular force balance can activate not only specific signaling pathways at the focal adhesion–integrin complexes but also induce changes in gene expression (163, 170–173). Therefore, according to this model, cells in all tissues may sense changes in gravity *via* alterations in the balance of forces distributed between their adhesion receptors and the cytoskeleton, rather than through direct activation of any single receptor molecule.

"What does this mean for how gravity influences cell and tissue development? Local distortion in the cytoskeleton appears to be common to all mechanisms of cellular mechanotransduction. Certain specialized mechanosensory cells, such as otoliths and sterocilia, utilize highly dense microstructures to induce mechanical strain in the cytoskeleton in order to experience gravitational acceleration. Other non-specialized cells may also feel the pull of gravity as a result of cytoskeleton distortion" (9). According to Ingber's model (9), the concept of a single gravity-specific receptor molecule and the idea that mechanical signals are transmitted equally at all points on the cell surface should be discarded. Instead, "gravity sensation should be viewed in the context of the structural complexity of living cells and tissues. In some cases the whole cell or even the whole tissue must be viewed as 'the' gravity sensor. In fact, gravity acting on the whole organism is a major contributor to pre-stress within individual tissues. When organisms are placed in microgravity, they experience an acute decrease in pre-stress on the macroscale which should produce corresponding changes in structure and mechanics at the cellular and molecular level" (166).

Cytoskeletal Changes during Microgravity and Role of RhoGTPases

In adherent cells, microtubules have a radial organization and actin stress fibers are anchored to the cell membrane. At these sites, cells can attach to their extracellular matrix *via* focal adhesion complexes. Actin is also localized at the cell border. Intermediate filaments form a loose network (15).

Several studies have demonstrated that microtubules self-organization is influenced by the direction and magnitude of gravity (174, 175). Therefore, when cells are exposed to microgravity, microtubules experience a disruption of the radial pattern (176) showing a perinuclear clustering distribution (177). This specific localization could lead to a reduced rate of chromosome segregation during mitosis.

Actin stress fibers are also affected by gravitational forces. Under low gravity environment, actin stress fibers are reduced in number, length, and thickness (178, 179). Actin is often redistributed and has either a more perinuclear or more cortical localization (180, 181). Focal adhesion proteins no longer align well with the stress fibers; instead, they appear as bigger clusters without radial orientation in the cortical layer, resulting in reduced cell spreading (15). All these changes may be explained by a decrease of RhoA activity. The absence of gravity increases the G-actin form, which reduces cofilin phosphorylation, and decreases focal adhesions and stress fibers formation (182). Microgravity has also an impact on intermediate filaments, which after 12 min in microgravity form clusters preferentially distributed around the nucleus (183).

The RhoGTPases, RhoA, Rac1, and Cdc42, are members of the Ras superfamily of small GTP-binding proteins that play key roles in cytoskeletal dynamics (184, 185). RhoA, for example, regulates focal adhesion assembly, stress fiber formation, and intracellular tension. Rac1 primarily controls actin assembly and formation of lamellipodia to ensure cell migration. RhoGTPases play an important role integrating mechanical and biochemical signals. Therefore, they seem to be at the forefront in cell adaptation to microgravity. According to Louis et al. (186), on $1 \times g$, cell tension is sensed through the cytoskeleton *via* microtubules, intermediate filaments, and actin stress fibers associated with focal adhesions within the extracellular matrix. These elements are controlled by GTPases RhoA and Rac1. During short-term exposure to microgravity, RhoA is inhibited to allow reduction in cell tension to adapt to the new mechanical environment. At the same time, Rac1 is activated to control peripheral actin polymerization. All these events lead rapidly to a rounder cell shape with disorganization of microtubules, stress fibers, intermediate filaments, and focal adhesions. Transcription may be also altered as nucleus shape is changed.

CONCLUSION

Understanding how living organisms have adapted to gravity is very complex, and the mechanisms responsible for this adaptation remain controversial. However, several of the studies reviewed provide some clues. For example, in an excellent review, Jamon

(72) shows that the vestibular response to microgravity is not only genetically programed but also depends on the exposure to gravity. "The gravistatic sensory system has a genetically controlled phase of development and a stimulus-controlled phase for fine-tuning synaptic terminals. Therefore, the level of gravity plays a critical role in fine-tuning of axons and is required for appropriate development of the projections from graviceptors to the brain and spinal cord" (72).

Moreover, several large-scale genomic-wide studies have been performed in various organisms to understand the impact of gravity on living organisms (146). These investigations have shown that cell proliferation, growth, differentiation, signaling, shape, and gene expression are all altered by changes in gravity. Interestingly, exposure to microgravity for a short period of time had no persistent effect on gene expression. In contrast, longer exposure can induce mutations in some genes that are stably inherited for several generations. Another important clue from mammalian experiments is that long-term adaptation to gravity may be also mediated by epigenetic changes in DNA.

It is now widely accepted that the cytoskeleton plays a role in sensing changes in gravity. However, the mechanisms by which living cells sense these signals and convert them into a biochemical response are still not well characterized. It is also controversial whether the forces induced by gravitational field on the cells are too small to induce any response (15). Given that gravity represents a modification in mechanical stresses encounter by the cells, the tensegrity model of cytoskeletal architecture (9) provides an excellent paradigm to explain how changes in the balance of forces, which are transmitted across transmembrane receptors and cytoskeleton, can influence intracellular signaling pathways and changes in gene expression.

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TN and JS-E have contributed to the revision of this article. JS-E has supervised and revised the final version.

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