Life in space isn't easy, even if you are green

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In order for terrestrial life to expand beyond the confines of our earthbound existence to bodies such as the moon or Mars it will have to tackle a barrage of stresses, some that it has encountered and adapted to over millions of years of evolution, but some that it will meet for the very first time. Whether reliable, sustainable biology-based (bio-regenerative) life support systems can be developed for long-duration spaceflight and extraterrestrial colonies has therefore become an important area of research. These systems would almost certainly centre on plants and microbes and so questions of how such organisms respond to reduced gravity and radiation become critical. Current research, using approaches ranging from gene expression and protein profiling to detailed growth analyses, suggests spaceflight triggers complex stress responses in these organisms, but that biology has a remarkable ability to cope with the life of a space alien.

To quote Konstantin Tsiolkovsky (1857–1935; one of the fathers of spaceflight): "The earth is the cradle of humanity, but mankind cannot stay in the cradle forever". We are at an exciting point in our journey to the stars. The International Space Station is providing us with a long-term presence in low earth orbit where we can experiment on what it means to inhabit space. In parallel, space agencies and commercial spaceflight providers are setting their sights on a manned presence on the moon, Mars and the spaces in between. If there is one constant in our earthbound lives, it is gravity; 1G is 1G and always has been. All organisms have evolved with this all-pervasive and unchanging force and so have had no evolutionary pressure to develop adaptations to changes in its level. Yet, in the 1950s, biology entered upon a grand adventure, space. Spaceflight has taken animals, plants and microbes to an environment outside the boundaries that have shaped their evolutionary history. The 'weightless' world of spaceflight triggers a myriad of changes in organisms from the cellular to the physiological. Even colonization of the moon or Mars will require life to thrive at ~17 or 38% of 1xG. Couple these effects with the other unique elements of leaving Tsiolkovsky's protective 'cradle', such as exposure to background galactic cosmic radiation, and one could justifiably ask whether biology can make the transition from its terrestrial roots. The answer to this question is that we are still at the very beginning of understanding how the space environment interacts with biological systems.

Increased access to experimentation in space is now beginning to uncover exactly what these new environments trigger in organisms ranging from humans and mice to plants and microbes. The effects on these latter two kingdoms are of particular interest as the mission distances being contemplated are immense and regular access to resupply ships from earth becomes less and less tenable as our sights move from our local surroundings to places such as Mars. Thus, not only will spacefarers have to endure the stresses of their alien environment, they will also have to be self-sufficient for all the nutritional requirements their metabolism demands.

Plants and space

Almost as soon as biological specimens were launched into space, plants were passengers on the rockets and plants have remained a major focus of space research to address two goals. Firstly, spaceflight provides a unique laboratory with which to dissect the role of gravity in modulating biological processes and to conduct weightless experiments that are impossible to perform on earth. In this case, investigating plant physiology, growth and development in space has been driven by curiosity, using this environment to dissect the most fundamental aspects of how plants work. The second driver for putting plants into space is more practical. Plants provide a wide range of services on earth, not least of which are delivering our food and oxygen and purifying our water. Therefore, another major goal for plant space researchers is understanding how to incorporate plants into life support systems that could sustain astronauts on long-term missions, so-called bio-regenerative life support. A further bonus to this role in sustaining the food, air and water supply of growing plants during spaceflight that has only just begun to be appreciated, is psychological support. Plants provide a living, growing link to the earth and a break from the highly engineered environments of the space vehicle. The key question that arises from these potential uses in bio-regenerative systems is how do plants respond to growing in space and could they be relied upon to provide critical elements of life support?

Plants do complete their life cycle in spaceflight. For example, in 2000, Mary Musgrave reported seed-to-seed growth of *Brassica rapa* over a 122-day mission on-board the Mir space station, although seed quality was compromised in the spaceflight plants. In addition, the lack of pollinators and the weightless environment of the spaceflight meant that the flowers had to be hand pollinated by the astronauts. The conclusion from many experiments is that plants do grow in space but the question remains, how well and reliably do they perform?

'Omics' and spaceflight

To help answer the question of how consistently productive plants are in spaceflight environments, we have an increasingly rich set of gene expression and protein profiling data from multiple space agencies and researchers for a range of plants grown in space including: Arabidopsis, rice, mizuna and even the fern Ceratopteris. NASA has also recently developed the GeneLab public data repository (Genelab.NASA.gov) which pulls many of these data sets together (along with many others on species ranging from mice and bacteria, to fruit flies and nematodes), providing an increasingly data-rich set of resources to mine for the molecular fingerprints of spaceflight responses.

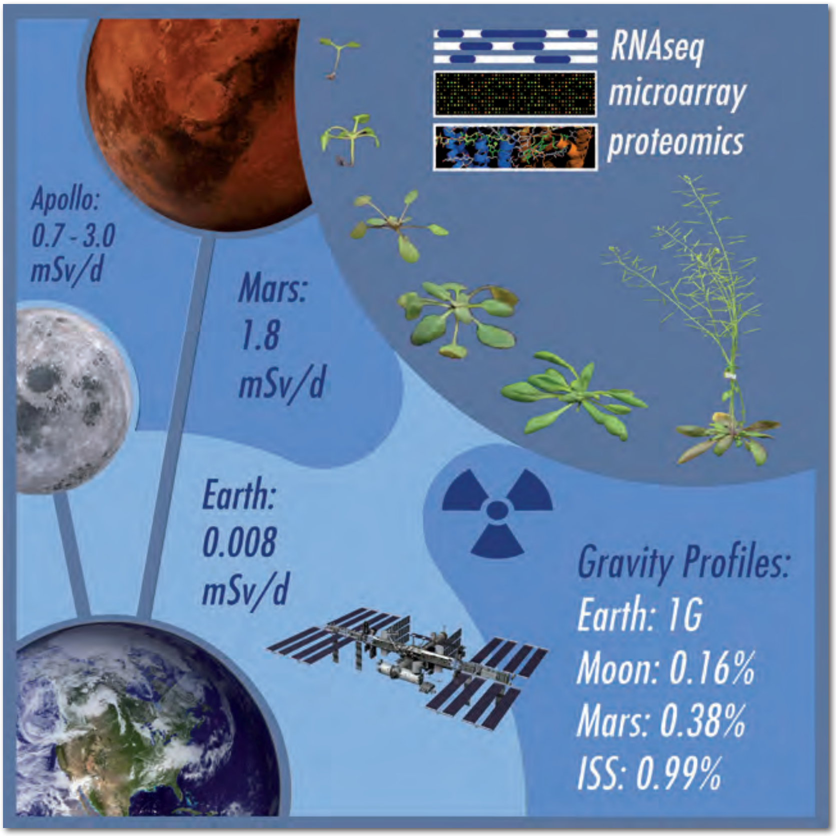
Some common themes are emerging from analyses of the plant spaceflight gene expression and protein profiling. There is a disruption of cell wall-related events that may well reflect the reduced mechanical loading from growth in a weightless environment. Thus, changes in the expression of wall-modifying enzymes are a common feature of gene expression analyses from Arabidopsis samples grown in space. Members of the peroxidase family were highlighted by Kwon and co-workers in 2015 as being down-regulated by spaceflight and alterations in transcript levels of these enzymes are also seen in many other spaceflight gene expression studies. Markers of oxidative stress are also regularly observed to be up-regulated across species as varied as rice, Arabidopsis and mizuna. Interestingly, in a spaceflight experiment where Arabidopsis cell cultures were flown on the Space Shuttle, up-regulation of molecular chaperones such as heat-shock proteins was noted. These proteins are thought to protect and repair cellular machinery from damage, so their increase likely reflects response to the cellular-level stresses that these conditions are triggering. Similar chaperone-related responses are seen at the whole seedling level, but the finding that these changes occur in cell cultures implies that this response is to an effect of spaceflight on some common cellular process rather than disruption of the directional gravity-sensing system that operates at the whole-organism level.

The story that emerges from these analyses is that a range of stress-signalling pathways are triggered by spaceflight. The major open question is how far these reflect adaptive responses to components of the spaceflight environment versus induction of inappropriate or random signalling networks in response to conditions that biology has never previously encountered.

Gravity: more than just up and down

The 'weightless' environment of spaceflight does disrupt many biological processes that rely upon directional sensing – yes, astronauts get disoriented and experience motion sickness when first flying in space and yes, plants lose their directional growth entrained to gravity (gravitropism) such that roots no longer grow down and shoots no longer grow up. This latter effect has been used as a tool to look for responses masked by the overwhelming directional cue of gravity on earth. Research by the Kiss laboratory has revealed how plants growing in space exhibit directional growth towards red light (red light phototropism) that is never visible on earth as the far stronger gravity response masks this light-driven effect.

However, gravity provides biology with much more than simply a sense of up and down. For example, on earth, gravity drives buoyancy-driven convection, i.e. warmer gases expand, are less dense and so rise due to their buoyancy, pulling in gases from below.



Spaceflight exposes terrestrial biology to a series of stresses including altered levels of gravity and increased radiation dosages that are outside life's evolutionary history. A range of technologies profiling patterns of gene expression (microarray, RNAseq) and protein levels (proteomics) are now being used to try to understand how organisms respond to this situation. Analysis of the model plant *Arabidopsis thaliana* grown in space is providing a window into the complex interplay of stresses that likely shape how biology acts in response to this alien environment. Radiation dosages are shown for the surface of the Earth, the trip to Mars and the range of exposures recorded for the Apollo astronauts during their moon missions.

In a weightless environment, buoyancy does not occur and so such convective mixing of gases is lost. On earth, temperature gradients are ubiquitous and so convective gas mixing is the norm. On the Space Station, without convection, steep gradients in gases build up and rapidly respiring tissues can deplete the oxygen around them. With no convective mixing, O_2 resupply is then limited to the rate of diffusion, severely restricting the O_2 supply. This effect is thought to lead to the generation of hypoxic zones around metabolically active regions of organisms, such as around rapidly respiring plant roots, which in turn negatively impacts growth and development.

Radiation: the great unknown

There is one further factor that contributes to 'space syndrome' once biology leaves the cradle of the earth's atmosphere and the protection of the planet's magnetosphere – radiation. Indeed, radiation damage has the potential to severely impede a sustained presence of terrestrial organisms away from their evolutionary home. The earth's magnetic field and the blanket of the atmosphere shields the biosphere from more than 99% of the harmful radiation produced by the sun and background galactic cosmic radiation. The average daily amount of radiation received by humans at the earth's surface is around 10 microsieverts, with most of this dose coming from geologic sources such as radon gas. In space, the radiation environment is much more pervasive and energetic. Galactic cosmic rays are produced by distant ancient supernovae and permeate space. Indeed, the Apollo astronauts reported seeing bright flashes of light every few minutes during their missions beyond the earth's protection and eventually these were determined to be from cosmic rays travelling through their eyes. Joining this background of galactic cosmic radiation are solar energetic particles, the 'solar wind', comprised of components such as protons fired from the sun during a coronal mass ejection event or as a result of distant stellar flares. How large is the radiation dose once leaving the earth's protective magnetosphere? While en route to Mars in 2012, the Curiosity rover's radiation meter was activated and recorded the radiation dosage of a true trip to the red planet. The journey delivered 0.33 sieverts, or about 90 years of background radiation on the earth's surface in a 253-day trip. Upon arrival, a Mars-bound astronaut would further have to cope with the fact that this planet has no magnetic field and solar winds stripped the atmosphere away millions of years ago, leaving a remnant of about 1% that of the earth. Thus, any organisms that are capable of making the journey would need to find shelter from the inevitable elevated radiation at the planet's surface. These realizations have spurred ideas including building Mars stations within the protection afforded by deep sheltered trenches on the Martian surface, such as Valles Marineris or the Hellas Planitia.

The key question now is how does this long-term radiation dosage from solar and galactic cosmic radiation affect biology? Once again, we are only just beginning to answer this question. The earth offers us so much radiation protection that performing the requisite long-duration experiments exposing biology to solar and galactic cosmic radiation for months to years have simply proven impossible. However, short-duration exposure experiments have been performed at facilities like the Space Radiation Laboratory at Brookhaven National Lab and so we do have some data on radiation responses to compare with spaceflight effects. The gene

expression data for Arabidopsis plants grown on the International Space Station show some changes expected for radiation response, but does not precisely mimic the patterns seen in the radiation experiments performed on earth. Confounding factors here may be that the low earth orbit of the Space Station is still within a large part of the protection afforded by the earth's magnetosphere, reducing exposure. In addition, it is unknown how closely the acute exposures provided in experiments on earth can mimic the biological effects of the prolonged lower level doses inherent in spaceflight. Whether radiation exposure will be just a technological hurdle to overcome or will become a permanent stress that life in space will have to somehow accommodate remains a critical question for our future away from the Earth.

Future perspectives

Humanity has long had Mars in its collective imagination and for centuries science fiction writers and scientific visionaries have postulated what might be found there and how the journey could be achieved. The flight to Mars will send explorers many millions of miles further than any other human has ever travelled. Research conducted on the International Space Station is now revealing many of the physiological consequences associated with such a trip but we are far from understanding just how terrestrial biology is affected by leaving the earth's protection. Over many millions of years, evolution has tailored organisms to thrive in the conditions of Tsiolkovsky's cradle. The challenge now is to understand whether we will have to somehow bring the conditions of earth with us wherever we travel or whether given enough biological insight and appropriate technology (both engineering- and biology-based) that the spaceflight environment will not be so alien after all. ■

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