Uncovering Genetic Changes to Explain the Effects Observed During Space Flight and Aging

Using customized microarray technology and state-of-the-art genetic techniques, Eugenia Wang has begun to uncover the genetic basis of microgravity-induced changes in the human body. She identified changes in the level of expression of ten genes in cells flown in space. This work could reveal genetic similarities between what occurs during space flight and aging, and eventually contribute to countermeasures for disorders associated with both space flight and aging, such as bone loss.

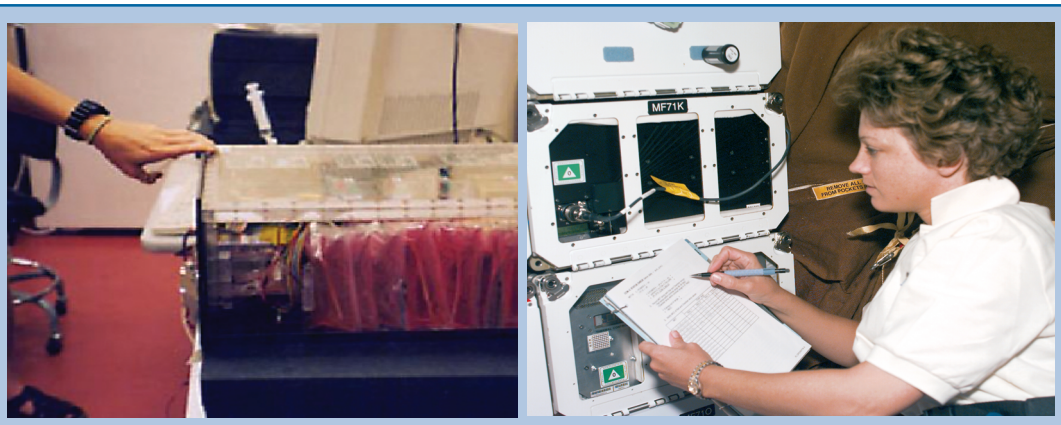
The changes in the body that humans and other vertebrates experience during space flight—such as accelerated bone loss, muscle atrophy, and cardiovascular deconditioning—represent both bad news and good news. The bad news is the effect of these changes on astronaut health. The levels of bone loss experienced during space flight, for example, are significant and, over the course of extended space travel, would represent a serious safety issue.

The good news is that the effects observed during space flight provide a unique opportunity to better understand the reasons underlying these changes—unique because these effects occur rapidly and therefore provide a promising model for study. Especially exciting is the possibility of improving our understanding of the changes all human bodies undergo as they age, since many of the effects of space flight parallel the effects observed during aging. What is different is that changes occur much more rapidly in space. An example is loss of bone mass, a disease associated primarily with osteoporosis and aging. Aging erodes bone mass gradually over many years, but space flight-related bone loss mimics this decline over a period of just weeks and months.

Eugenia Wang, Ph.D., of the University of Louisville has used the most recent genetic technologies to begin to investigate the genetic basis of the changes observed during space travel and to look for genetic similarities between the effects of space flight and aging.

Too Many Genes

When an organism reacts to a stimulus, in this case the environmental stimulus of microgravity, individual genes respond by altering their level of expression. Known as transcriptional regulation for gene expression, genes either control other genes to alter their expression and/or change their own levels of expression. This is the very first step in the eventual production of the corresponding proteins that ultimately alter cell function.

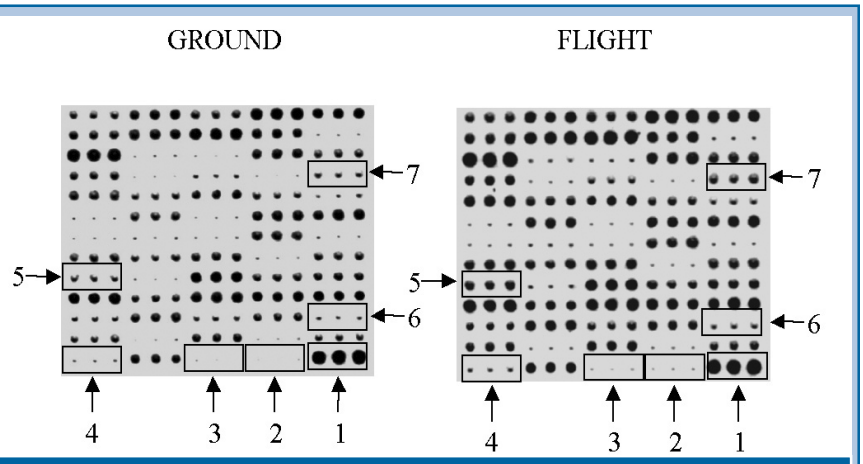


(Left) The sealed cell culture payload being readied for its STS-93 launch. (Right) Astronaut Eileen M. Collins, STS-93 mission commander, checks the status of the Cell Culture Module on the middeck. (NASA photo S93-E-5069.)

Of the thousands of possible genes to examine, Wang chose 202 genes belonging to three key gene families. The families of genes Wang selected are those that are activated first and that then affect the activity of genes that act later. These genes are the first to react in situations that are stressful or damaging to a cell. As Wang says, "These are sort of generic genes that act as a first line of defense for all tissue. They are specifically susceptible to oxidative damage, mechanical stress, and changes from normal gravity to hypergravity to hypogravity," and are potentially related to both adaptation to space flight and to aging.

To identify the genes expressed in both flight and control cells, Wang designed microarrays, also known as genechips. Microarrays are powerful tools that allow hundreds or thousands of genes to be screened in parallel. Wang's microarrays focused specifically on the families of genes she had selected earlier, using novel development techniques to heighten their sensitivity.

Wang flew cultures of human fibroblasts—cells involved in the formation of connective tissue and commonly used for research on cellular effects including aging—on the STS-93 Space Shuttle mission in 1999. The cell cultures, which were composed of young cells, were exposed to microgravity for almost five days in a device called the Cell Culture Module. Once the cells were returned to Earth, Wang compared gene expression in the flight cells to control cells.



Example of microarray analysis of gene expression. The ground control sample is on the left side and the flight sample is on the right. Numbers 1-3 are internal controls. Numbers 4-7 represent four different genes of the tumor necrosis factor family. Expression of these genes was altered following space flight, as seen in the more intense dots in the flight sample.

Identification of Genes Altered in Space Points the Way to Future Research

Through the microarray analysis, Wang found ten genes that exhibited altered expression as a result of space flight. Eight of the genes belong to the tumor necrosis factor gene family and two belong to the interleukin gene family. Most of these genes are involved in either the regulation of bone density—and therefore are likely involved in space-induced bone loss—or in the development of proinflammatory responses (stress-related).

As stated in her 2002 *FASEB Journal* article, Wang "identified a complex series of changes in response to microgravity," changes that "may underlie a mechanism attempting to counteract the bone loss occurring as a result of space flight."

Next steps for this research will involve looking at changes in expression of later-acting genes to get a more thorough picture of the genetic effects of microgravity by identifying the signal pathways involved. Wang also plans to determine whether the genetic changes exhibited during space flight also occur as a result of normal aging. These further studies may lay the groundwork for developing countermeasures for microgravity-related and aging effects on the human body, especially bone loss.

As Wang states, because of the apparent time compression of space flight effects compared to aging effects, "any therapeutic treatment to combat microgravity-induced degeneration could be easily adapted to the attempt to deter, delay, or reduce the burden of age-dependent debility" (*FASEB Journal* article, 1999).

Multiplying a Finite Resource

One of the challenges of conducting research in "outer space" is the strict constraint placed on "inner space." The volume allotted to any particular experiment on a spacecraft is very limited. This meant that the size of Wang's cell cultures had to be very small, limiting the amount of material that would be available for postflight analysis. However, by using the latest techniques in biotechnology, she was able to generate sufficient material for her immediate needs and create an archive of material containing the valuable space flight genetic data for future experiments by her or other researchers. Wang did this by creating a cDNA library, which allowed her "to convert the limited biological material to an inexhaustible platform."

A cDNA library is a pool of DNA synthesized using RNA as a template. The synthesized DNA "matches" or is complementary to the RNA. The RNA from the flight cells was used to create the cDNA. The cDNA can then be cloned or duplicated by inserting it into a bacterium that is cultured, producing an unlimited supply of the genetic information from the cells flown in space. A corresponding cDNA library was also created for the control cells.

References

1. Semov A; Semova N; Lacelle C; Marcotte R; Petroulakis E; Proestou G; Wang E. Alterations in TNF- and IL-related gene expression in space-flown WI38 human fibroblasts. *FASEB Journal* 16(8):899-901, 2002.
2. Wang E. Age-dependent atrophy and microgravity travel: what do they have in common? *FASEB Journal* 13 Suppl:S167-74, 1999.