NASA FUNDAMENTAL BIOLOGY

SPACE GENETICS

Report on flight readiness of model organism
Saccharomyces cerevisiae

19 April 2001
NASA Ames Research Center

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Executive Summary
Working Group for *Saccharomyces cerevisiae* Space Studies

The *Saccharomyces cerevisiae* Working Group met on 19 April 2001 at the NASA Ames Research Center to determine the scientific value and feasibility of flying the yeast, *Saccharomyces cerevisiae,* in space. The participants were experts on this species, on cell and molecular biology, and on bioinformatics from major universities, including world experts on the topic (see participant list on page TBD). The consensus of the Working Group is as follows.

Saccharomyces cerevisiae is one of the most important organisms, if not *the* most important organism, to fly in space in the near term. It is the single celled organism that behaves most like human cells and many aspects of human cell biology were originally worked out using yeast. Flying this organism in space provides crucial foundational information relevant to basic science as well as space medicine.

It is the best understood eukaryotic organism in history. It's full genome is known; most of the functions of the genome are known; most of the life cycle is exceptionally well characterized.

The hardware needed to conduct definitive studies on yeast is available now through NASA Centers for Commercial Development. The fixatives needed to preserve the inflight data are flight qualified.

Critical pioneering investigations can be implemented in middeck class allocations. Important pieces of the investigation could also be carried out as piggybacks on other investigations and as "Minutemen" where *Saccharomyces cerevisiae* is incorporated on a flight at the last minute. Even PICOSAT free flyers are possible vehicles for yeast studies.

The group recommends implementation at the earliest opportunity. This study could be ready to fly within the year at relatively low cost.

The results are expected to be definitive and of exceptional quality and scope. This would serve to demonstrate the value of the space environment for important new discoveries in cell and molecular biology.

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Advisory Group for *Saccharomyces cerevisiae*: SpaceStudies

19 April 2001

NASA Fundamental Biology Program
NASA Ames Research Center
Bldg. 244, Conference Room 103

AGENDA

9:00	Welcome and Charge to the Group Mel Averner, Program Manager, Fundamental Biology
9:30	Constraints of Spaceflight Research (Rita Briggs)
9:50	Available Flight Hardware (Rita Briggs)
10:10	Experimental Controls for Space Studies (Timothy Hammond)
10:30	Experimental Conditions for Yeast Studies (Roymarie Ballester)
10:50	Recommendations for Flight Studies, for example, • types of baseline data collections • culture conditions • inflight protocols • postflight analyses
12:00	Lunch
1:00	Recommendations for Flight Studies (continued)
3:30	Wrap-up

Working Group for *Saccharomyces cerevisiae* Space Studies

REPORT FOR THE RECORD

"The key to every biological problem must finally be sought in the cell,"
biologist E.B. Wilson, 1925.

Yeast, especially *Saccharomyces cerevisiae*, is a surprisingly important organism to study in space. Surprising, because it is a fungi, and a single celled fungi at that. However, humans and yeast have a close relationship that extends amicably back in time for thousands of years. *Saccharomyces cerevisiae* , more commonly known as Brewers yeast, is the organism responsible for turning grapes to wine, hops to beer, and wheat flour to leavened bread. We are old friends.

Recently, Brewer's yeast became extremely important to us, not just for providing the drinks at the party, but for providing some of the most necessary insights in the history of science on how animal cells -- and especially how human cells -- work. From this knowledge, a significant portion of the biotech revolution was spawned.

The Human Genome Project revealed that humans and Brewer's yeast have an astonishing number of genes in common (SPECIFY). More importantly, these genes carry out many of the same protein related activities in each species. Brewer's yeast is a major pathfinding species for the Human Genome Project and is one of the two best understood organisms in the world (the other being *E.coli*). For these reasons, flying *Saccharomyces cerevisiae* in space is a high priority in Space Genetics.

To explore the feasibility and cost of conducting studies of Brewer's yeast in space, a working group was sponsored by NASA's Fundamental Biology Program and convened 19 April 2001 at the NASA Ames Research Center. The goal was to determine what studies on yeast could be conducted within the next 3 years that could provide important – even foundational -- insights on major biological issues about life in space. Emphasis was placed on investigations that could yield a high science value within the accommodations and constraints of the early Space Station era.

The consensus of the Working Group was that yeast is a very high priority species to fly in space and that a pioneering yeast investigation could be ready for spaceflight implementation within a year using available flight opportunites and flight hardware. The results are expected to provide the fundamental underpinning for understanding animal cell biology, including human biology, in space.

The Working Group confirmed that all the hardware needed to support very high quality research on this organism in space is on the shelf and ready to fly in middeck class accommodations. The investigation can be implemented very inexpensively and within months of the commitment to proceed. The organism has been cloned and microarrays are available for rapid analysis. (Analysis of gene expression of an organism's entire genome can be completed within hours). An NRA can be used to solicit investigators postflight to analyze the remarkable amount of data that can be generated from a single middeck flight. This significantly increases opportunities for community participation in space biology research while reducing the front end costs to develop an investigation. Investigators can be brought in when samples are ready for them to analyze.

In short, this is a pioneering investigation on many levels that is ready for implementation now.

Discussion

The excitement over Space Genetics in general and *S. cerevisiae* in particular is based on a historic convergence of three technological revolutions: in biotechnology, information technology, and miniaturization technologies. These can now be coupled with a sudden increase in habitable flight volumes provided when the Space Station joined the Space Shuttle as a space flight research asset. Twenty years of experience in the development and commercialization of space laboratory equipment provides the necessary research equipment on the shelf. New developments in freeflyers, such as Stanford University's PICOSAT program amplifies research opportunities.

The resulting merger allows us to conduct biological research in space that can be expected to have the same magnitude of impact on space biosciences that the Hubble Space Telescope had on astronomy. It heralds a major paradigm shift.

This is because modern biotechnology allows us to watch and manipulate the inner workings of the cell. The cell is the basic unit of life as the atom is the basic unit of matter. What distinguishes one species from another and one individual from another is the set of biochemical instructions encoded in the genome. We can now read the instructions and, in yeast more than any other animal or plant cell, understand what the instructions mean to the organism's health and reproductive success.

The genome is the sum of all genes an organism possesses. The central dogma of biology is that genes direct the production of proteins. Differing in ways subtle and gross in their size, composition and shape, proteins are responsible for most of the activities of life. Indeed, proteins determine the shape life takes, its robustness, and its success at reproduction. Proteins can be structural, such as the actin and myosin proteins that produce muscle; metabolic, such as insulin which enables cells to use food energy, or regulatory such as the transcription proteins that turn on gene expression. The complex ballet of genes and proteins enables all the diversity of life that we know on Earth. Because life on Earth started from a common ancestor, the basic rules of biology that apply to a yeast, apply to a human to an extent great enough to use yeast to probe into some of humanity's most intractable biological issues.

By knowing what genes cause the productions of which proteins, the book of life can be read with exquisite precision. Even better, the value of the data improves over time as new data are added.

The awesome power of yeast genetics is partially due to the ability to quickly map a phenotype producing gene (a gene that produces a measurable effect) to a region of the *S. cerevisiae* genome. For the past two decades S. cerevisiae has been the model system for much of molecular genetic research because the basic cellular mechanics of replication, recombination, cell division and metabolism are generally conserved (very similar because of a common evolutionary heritage) between yeast and larger eukaryotes, including mammals and humans.

For these reasons, understanding the space genetics of *Saccharomyces cerevisiae*.is a strong candidate for priority allocations within spacecraft in the immediate future.

The *Saccharomyces cerevisiae* Working Group noted the following:

- **Brewer's yeast is one of the most important organisms to fly to understand the response of terran life to space.** Because we share so many genes in common, the results are expected to be relevant to the human condition, both in space and on the ground. Understanding these molecular mechanisms in space is one of the first steps needed to remove the biological barriers to human exploration of the solar system. In addition, the space environment is so novel from an evolutionary point of view that the growth of yeast in space may reveal features of terrestrial life that cannot be seen on Earth. More than any other single organism, yeast can confirm the validity of this statement.

- **Results are relevant to plants, animals, and humans as well as to other microbial species**. Yeast reproduce asexually through mitosis, as do most animal and plant cells. They also reproduce sexually through meiosis, as animal cells do – including human reproductive cells -- during the first step of embryonic development. The study of yeast in space provides insight into the effects of the space environment on these most critical cell functions.

- **Cause and effect relationships can be determined through the design of multifactor experiments**. Genomic, proteomic, metabolic and structural information could be obtained in a correlative manner (see Figure 1) on *Saccharomyces cerevisiae* in space on middeck level accommodations. This allows us to identify major elements in the adaptation of life to space in the sequence from the cell's initial detection of the space environment to its signal for a change in gene expression to the result over multiple generations. In yeast, multiple generations are achieved within a day.

- **The necessary hardware is on the shelf** (see Appendix 1), so investigations could begin now on middeck class accommodations. The major challenge is in preserving the inflight data, but for most of the investigations examined, the fixatives needed have already been flight qualified.

- **If any specific genes are necessary for adaptation of yeast to space, these can be identified.** Yeast has approximately 6284 genes in its genome. The same number of mutant strains of *Saccharomyces cerevisiae* exist. Each individual strain has one gene of the total *S. cerevisiae* genome deleted. These are called deletion mutants. By flying a mixed culture of deletion mutants in space, researchers can determine which if any genes are essential for the adaptive response to space and which are not. (This concept is proprietary to Dr. Victor Stolz, NASA Ames Research Center).

- **A new paradigm may be revealed, or a new control strategy for other biological investigations may be discovered.** If yeast, which is a small cell, is sensitive to the space environment, a new paradigm for understanding life in space would be needed and a new realm of discovery would be opened for exploration. If yeast is not sensitive to growth in space, this information would be useful and perhaps even preferred. The ability to fly an organism that is not sensitive to microgravity but is sensitive to other environmental variables in space would act as a powerful control for other biological research in space.

- **The results are expected to be definitve.** Because yeast can be flown in a dormant, dried state, the experiments can be started in space. This eliminates the confounding variables of launch stress on the interpretation of results. It also means that yeast can be prepared to fly as a "Minuteman", an opportunistic last minute addition to a mission. Further, changes in gene expression are often diagnostic of the cause of these changes, especially in an organism as well understood as Brewer's yeast. In this way, the gene expression data act as their own controls. Yeast is so easy to handle and so much information can be obtained from even small volumes, that piggyback investigations, where only part of a middeck cell culture system is available would yield very useful results.

– **Minuteman and piggyback investigations are possible.** A preliminary concept shows that useful data can be obtained when cell biology investigations of this kind are carried out as only part of a middeck allocation. The results can be importantly contributory or confirmatory under these circumstances. However, they are unlikely to be comprehensive enough to achieve the discoveries necessary for a paradigm shift.

– **Look for as many flight opportunities as possible.** Consider implementing yeast investigations on commercial flights with groups such as Space Explorers, Inc. and pharmaceutical or other companies. Consider the use of free flyers like PICOSATs and TransHab derivatives to fly outside the Van Allen belts for radiation studies.

The Working Group recommended the following experiment design concepts for yeast studies:

- **Freeze-dried (lyophilized) yeast should be launched, then activated in space** by adding nutrient media. This eliminates the confounding variables of the launch stresses: acceleration, vibration, acoustic.

- **Time course changes are essential.** The experiment should be designed to fix data at several different time points: life is a movie, not a snapshot. To establish the initial sequence of events, only about 4-5 days of data are required with samples collected at roughly 6 hour intervals (this is somewhat flexible).

- **Confirmation is essential.** Repeatability is the essence of science. At least two separate flights will be required, although the confirmation may be able to be carried out on a piggyback basis.

- **Deletion mutants should be flown as soon as possible** to determine which genes are essential for space adaptation.

- **Both sexual and asexual reproduction should be studied.**

- **Fly both haploid and diploid cells and also 2 different yeasts**, such as *Candida albacans* and *Schizosaccharomyces pombe*, to triangulate the analysis of cells in space.

- **Various fluorescent proteins should be used to examine metabolic and structural processes** including: cytoskeleton, transcription factors, gene products, organization of cell organelles, budding, reproduction, nuclear orientation, spindle charateristics, organization of membrane structures, mitochondria, etc.

- **Gene expression, proteomics, gene regulatory mechanisms, and structure/metabolism should be examined together on a single flight.**

With the exception of the necessary confirmation flight, obtaining all of the data described above could be carried out in 1 and at the most 2 middeck lockers on a single flight. After this, the array of possible followup studies becomes so vast that either a workshop or an NRA is recommended to define them.

A new strategy for space flight research can be implemented with this investigation. The techniques for achieving high quality cultures are standardized and can be achieved in space. It was noted that an important result of these yeast investigations could be to demonstrate to a skeptical science community that data of exceptional quality for molecular biology investigations can be obtained from space research facilities. Yeast is such a well known species, that the adequacy of the data will be immediately apparent to most of the cell and molecular biology community.

The Workshop participants also observed that there was little to be gained by using an NRA to select scientists to guide the upfront development of the yeast investigations. A better idea is to develop a set of standardized methods, fly the experiments, and release an NRA for the postflight analysis of the samples. Developing the yeast cultures for flight could be carried out by inhouse molecular biologists. The investigations can be carried out on commercial flight hardware and samples fixed inflight. The samples can be analysed postflight by commercial or university vendors. Following this, a large data mining effort would be mounted to assist in interpretation of the data and translation of the resultant information into a useful application. An NRA could be released for the critical interpretation step. The rest of the processes could be streamlined and commercialized for low cost execution.

Summary of Key Points:

The consensus of the group was to confirm the value of a near-term comprehensive study of yeast in space, initially and with priority on *Saccharomyces cerevisiae*.because:

- It is the best understood eukaryotic cell on Earth and was the first eukaryotic organism sequenced by the Human Genome Project.
- It has a large number of genes in common with humans. Indeed, it is the most like human beings of any single celled organism.
- It's full genome is known, and gene chips are readily available for rapid analysis.
- Over 60% of the genes have been matched .
- Deletion mutants for every gene exist.

They confirmed that:

- this species has already flown in space and is easy to grow,
- preservation of the inflight data could be achieved using fixatives that are alreaady flight qualitied,
- the flight investigation could be developed and flown within weeks of an opportunity.
- The flight hardware is on the shelf: in fact several different systems are available.
- Sophisticated investigations require only 1-2 middeck equivalent lockers per flight.
- Important investigations can even share facilities with other investigations, enabling some powerful "nook and cranny" science. Indeed, standard cultures could be maintained at KSC ready to fly at the last minute.

Conclusion:

The Human Genome Project has been called reading the book of life. If NASA decides to read the book of life in space, then *Saccharomyces cerevisiae* must surely be in the first chapter. Functional genomic research on this organism fits the definition of a pioneering investigation in space.

The good news is that the pioneering investigations can be carried out now, flight opportunities are frequent, the cost to conduct the investigation is cheap, and the richness of information is exceptional. The better news is that the value of the data will actually improve over time as new bioinformatics tools are applied to amplify the data yield and as later investigations build on the first ones to fully characterize a life form in space. The best news is that this organism, because of its size, stands at the threshold of detectability for space flight events. If *Saccharomyces cerevisiae* is sensitive to microgravity, a new paradigm for life in space has been discovered. If it is not sensitive to microgravity, then *Saccharomyces cerevisiae* can serve as the long sought control organism that can be used to detect other environmental perturbations in the space environment that might have a biological effect.

Figure 1. Schematic of Molecular Biology of Life in Space

Bacteria, yeasts, Drosophila, C. elegans, plants, fish
Human and animal tissue cultures
Cell lines
Embryos
Eventually human and mammal subjects

Time course, cell cycles, life cycles, n generations

Research platforms	Space ⇒	Δ organism/ environment interface	Signal ⇒	Δ gene expression	mRNA ⇒	Δ protein complement	⇒	Δ metabolism	Δ structure
Shuttle Cells, tissues, adaptation **Early ISS** Small organisms life cycles, tissue aggregates **Later ISS** Larger organisms, multiple generations, evolution **Planets**	Start experi-ment in space via freezing, dormancy adding nutrients in flight	optimizes suspension cultures fluid dynamics theory, modeling and optimization studies imaging and monitoring	- gravity - shear - density-driven forces - coriolis -turbulence -convection + radiation	10Kgenes at a time use specimens where full genome and microarray are available leverage and contribute to databases	Fix + refrigerate or Freeze @ (-80°C) Enables cDNA library	Fix, snap freeze @ (-80°C), store @ (-120°C) ----------- fluorescent proteins analyses scale with available sample correlate with gene array data		Substrates for different enzymes Time samples	Fix for electron microscopy

Evolutionary relationships

- REFERENCES:

Basic Tutorial on Yeast: http://genome-www.stanford.edu/Saccharomyces/VL-what_are_yeast.html

Comprehensive information on Yeast: http://genome-www.stanford.edu/Saccharomyces/VL-yeast.html

Previous investigations on Yeast in space
http://web-x.arc.nasa.gov/ImageDB/experiment_images.cfm?ID=60
http://web-x.arc.nasa.gov/ImageDB/experiment_images.cfm?ID=145

Why you use yeast, flies, worms, and mice to understand human cell biology:
http://www.hhmi.org/annual/research/fwhg.htm

Previous investigations of gene expression changes in space
http://physiolgenomics.physiology.org/cgi/content/full/3/3/163#BDY

Cell Biology Hardware for Space Flight Studies:
http://astrobiology.arc.nasa.gov/genomics/technologies/available_hardware.html

Appendix 1 Hardware List