

Saccharomyces cerevisiae

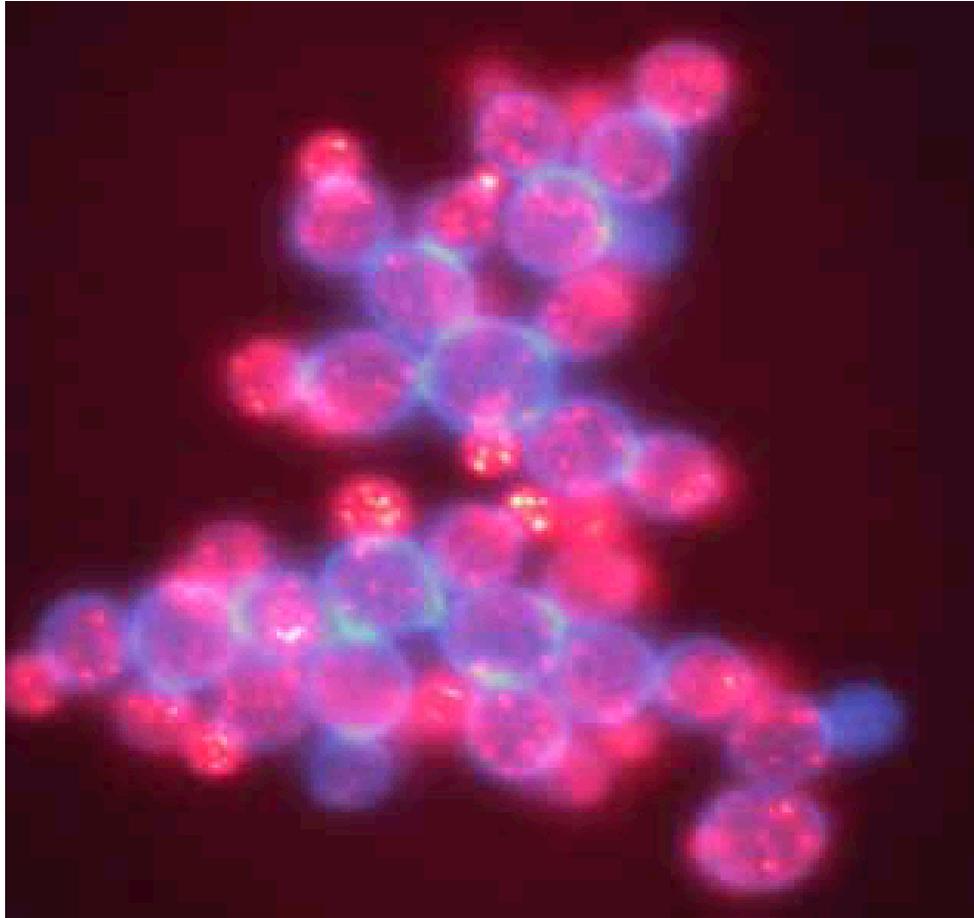


Photo by Eric Weiss; submitted by David Drubin
http://ls.berkeley.edu/divisions/bio/gallery_mcb/yeast_mutant.html

NASA FUNDAMENTAL BIOLOGY

SPACE STUDIES

Report on *Saccharomyces cerevisiae* as a Model Organism for Space Studies

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 evaluate 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; they came from a variety of universities, companies and from NASA Ames Research Center. The consensus of the Working Group is as follows.

Saccharomyces cerevisiae is an excellent candidate for near term research in the space environment. It is a single celled, nucleated organism that, in many ways, behaves like a human cell. Investigating the metabolic behavior of this organism in space, using recently developed, highly sensitive methods of genomic analyses, will ultimately provide crucial foundational information relevant to basic science as well as to biomedicine.

Saccharomyces cerevisiae is a well understood eukaryotic organism whose full genome is known. In addition, most of the functions of the genome are known, and its the life cycle is exceptionally well characterized.

The hardware needed to conduct definitive studies on yeast in space is available now through commercial sources such as NASA Centers for Commercial Development. The materials and fixatives needed to preserve the data collected during space flight are flight qualified.

Critical exploratory investigations can be implemented in middeck-locker sized units. Alternatively, important aspects of in-space investigations could also be carried out using small, autonomous packages that are launched attached to other, unrelated payloads, as “piggyback” payloads, or as “Minutemen” where a *Saccharomyces cerevisiae* study package would be added to a payload at the last minute. Even the use of PICOSAT free flyers is a possible method for getting yeast studies into the space environment.

The group recommends implementation of in-space *Saccharomyces cerevisiae* exploratory studies at the earliest opportunity. Based on information available at the time of the meeting, it was determined that such investigations could be ready to fly within 8 months, and at relatively low cost.

Because of the simplicity of the organism, of the flight hardware, and of the methods of analyses, the results of such space flight investigations are expected to be definitive and of exceptional quality and scope. The program projected would serve to demonstrate the value of the space environment for important new discoveries in cell and molecular biology.

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<http://www.operon.com/>

<http://www.operon.com/search.php?OPERONSESSID=2d394a959df648a500ecef2b470f236&search=seidel>

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http://ls.berkeley.edu/divisions/bio/gallery_mcb/yeast_mutant.html

Advisory Group for *Saccharomyces cerevisiae*: Space Studies

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
- 9:10 Introductions
- 9:30 Available Flight Hardware (Rita Briggs)
- 9:50 Experimental Controls for Space Studies (Timothy Hammond)
- 10:10 Experimental Conditions for Yeast Studies (Roymarie Ballister)
- 10:30 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

***Saccharomyces cerevisiae* Advisory Group Meeting**

MINUTES

Commencement of Meeting: The meeting started at 9 am. with personal introductions of active participants: Roymarie Ballister, Richard d Boyle, Rita Briggs, Clark Glymour, Tim Hammond, Lynn Harper, Chris Seidel, Viktor Stolc, Eric Weiss. Others (see page 3) joined the meeting at later times.

Charge to the Group: Dr. Mel Averner, Program Manager, Fundamental Biology Research Program, who sponsored this meeting addressed the group as follows: “We have been flying living systems in space for several decades and have observed physiological changes. We need to better understand these complex changes. Tim’s experiment showed unique gene changes in response to space environment. Other experiments with fibroblasts and also our archive tissue bank with 10-year old tissue have given us the evidence to support an aggressive program..... There is a body of opinion that yeast is too small to show space effects but that theory has not been tested. That is why we have asked you to this workshop - to advise us on using yeast as a model to conduct space studies -----.

Presentation of Support Hardware:

Four different types of hardware that are flight qualified and potentially can maintain yeast experiments (attachment 2) were described. (1) The Bioreactor developed by the European Space Agency is specific for yeast experiments but requires manual intervention by the crew. This hardware can be acquired through ISLSWG - International Life Science Working Group consortium. (2) The Isothermal Containment Module developed by Bioserve has a good flight history, is fully automated, and can provide ample samples. (3) The Biofac/Biotech processing facility, by SHOT, Inc., also is fully automated with possible 1-g in-flight controls and a good flight record. (4) The Avian Development Facility, also by SHOT, is another possible candidate but has not been tested with anything other than avian eggs.

A list of fixatives that are flight qualified also was presented. The group indicated that fixatives required for yeast experiments were included in this list (e.g. formaldehyde).

Potential freezing capabilities were discussed including: (1) passive freezer (-195°C) with 21-day duration, (2) -20°C freezer (OSRF) which flew on STS-95 and its upgrade ARCTIC, and (3) -80°C freezer (MELFI) to be delivered to the ISS in early ‘02].

Discussion/Recommendations

- The BioServe and SHOT hardware were recognized as having the advantage of being fully automated.
- The need to avoid crew interaction and having the right controls were stressed.

- It was suggested, since it takes so long to get flight opportunities, that we should aim for multifactor experiment designs (multiple conditions).
- Viktor Stolc proposed that the Yeast Deletion Project would serve the multifactor idea since one can examine all gene deletions in one vessel. In this way, every single gene can be evaluated as well as growth rate as a function of time.
- Lynn Harper also proposed that being ready to fly at a moment's notice (Minuteman Status) increases flight opportunities.
- Fixation of yeast was discussed; not sure whether sodium azide would work to stop cell division but dropping temperature down to 4°C should.

Presentation on Experimental Controls:

Tim Hammond gave an overview of his two kidney cell experiments using multiple ground controls – 1 hardware control, 1 vibration, 1 hypergravity (3g), 1 Rotating Wall Vessel. Gene arrays demonstrated some changes specific to each of these conditions (see <http://www.som.tulane.edu/astrobiology/microarray/> - under the scatter diagram are hot links to the top 100 genes that changed after 6 days in space both up and down compared with a static control.

Discussion/Recommendations:

- The question of where to set the change in gene expression was raised. Tim said he reported genes, which had at least a 2-fold change in expression.
- The right controls are dependent upon the type of experiment flown but in general one has to consider things like launch slips
- There were questions regarding the radiation environment. How is radiation controlled for, how relevant is cell response to the whole person, and how does radiation vary with time? A lengthy discussion followed on the difficulty of radiation controls and the poor characterization of secondary radiation inside the ISS. In summary, it was noted that shielding – which is very heavy and imposes volume and weight problems – is the only way to protect against radiation, and passive dosimeters are the only way to measure exposure; the previous radiation program on MIR looked at replication; radiation studies on the ground cannot evaluate the synergistic effects of the different kinds of radiation; and the major radiation issues will occur when we go beyond the Van Allen Belt and not on the ISS.

Presentation on Stress Response and studies with the CCU:

Roymarie Ballister described her work on stress response in yeast. She has identified a group of proteins believed to be stress receptors. Stress response has been highly conserved during evolution and all cell types use similar pathways. Two primary pathways were discussed – MAPKinase and Heat Shock Response pathways. It was Roymarie's opinion that the latter would be turned on in response to the space environment because she believes that the group of proteins identified act as mechanosensors. These proteins also regulate actin cytoskeleton and, therefore, helps maintain cell shape – another parameter to look at in space. She wondered whether the

mechanism of shear stress explained the effects of centrifugal forces; when she centrifuged a GFP-fused transcription factor, that factor translocated to the nucleus . Protein translocation to the nucleus also happens with heat-shocked cells.

As a consultant to the SSBRP Program at Ames Research Center, Roymarie has been evaluating the growth rate of yeast and data collection techniques for the Cell Culture Unit. Some findings suggest that:

1. Growing cells at low temperature is not a good idea because you select for low temperature mutants
2. Glycerol is not a good alternative to carbon-source feed because, again, selection occurs
3. Cultures should be started at $10^3 - 10^4$ cells to avoid overgrowth. The drawback is that you need a person (inflight?) to do it.
4. Different genes are switched on at different growth stages, e.g., stationary vs. log phase.

Discussion/Recommendations:

- The point was made that multiple generations are important for evolutionary studies but are not necessary for many experiments. Yeast has a 2-hour doubling time. Experiments designed for ISS are long-duration.
- Since the CCU is not developed for flight yet, Tim suggested that we consider flying an experiment similar to the one flown by a PI (Augusto Cogoli) and his colleagues in the European Space Agency - (see Walther et. al, Enzyme and Microbial Technology 27 (2000) 778-783). These researchers used a bioreactor designed for yeast experiments and found that microgravity and cultivation conditions (unstirred) affected the specific bud scar positioning. Metabolism and cell morphology were unchanged. They also used continuous flow system and extracted yeast cells, not just effluent, thereby regulating yeast culture.
- Continuous flow system – this can be used for yeast
- Synchronization - this is not a problem, yeast cells establish quickly.
- GFP-labeled yeast - can be cooled and brought back
- Sample volume - only a few ml. are required. Initially sampling should occur every 6 hours, then 1x/day.
- Replicates - don't need for Yeast Deletion Project. Can use 4 different fluorescent proteins. Compare different strains of yeast – a common expression would suggest those particular genes have common function in space. Should do expression profile and deletion profile.
- Redundant genes – yeast has some redundancy
- Live cell analysis - look at GFP-fusion protein e.g. actin skeleton – label some substructure.
- Imaging - hardware developed by both BioServe and Walter Reed Armed Forces Institute of Research have some real-time imaging capabilities. Problem is what focal lens to use. Consider using CCD camera. Can study

membrane traffic (endocytosis); problem is assay works very quickly; have to image 5 minutes after adding GFP.

- Structure or Genomics – what is more important? Structural studies can be done using genomics – tag structural proteins with GFP or GST (glucokinase transferase ?). Problem is that we don't know which cells in population are expressing GST or fusion kinase; system is not ready to use yet.
- Tagging genes – consensus that yeast is the best model.
- Top level analyses could include membrane organization – actin, vacuoles, golgi, nuclear orientation. Fix with formaldehyde, half-hour later wash with PBS, remove wash and refrigerate. Continuous fixation interferes with some organelles/features. Need hardware capability to change out liquid medium, wash and fix. Fix enough cells for archiving.
- Last minute opportunities – Minuteman – also can increase yield. Have experiments ready to-go, store at KSC, do competitive post-flight NRA.
- Hypothesis vs. Observational studies. Lengthy discussion on this topic.
- Yeast is a well-established workhorse. Yeast is the simplest organism most like us. Many geneticists have switched to using yeast as their research model.
- Sensitivity to microgravity - worst scenario is that yeast is not sensitive to microgravity. If this is the case, then it becomes an ideal control.

Why Yeast is a good model for space studies

- Easiest genetic organism that you can manipulate and which gives information relevant to eukaryotes and humans.
- Yeast and people have a number of genes in common. Specifically, kidney cells that flew have genes in common with yeast.
- Full genome is known; every gene has been deleted. Every gene deletion is a potential mutant which provides functional information.
- Yeast is a good model to study interaction between genes
- Easy to grow in space and costs relatively low
- Has been shown to be affected by space conditions e.g. Cogoli saw a really significant change in the specific bud scar positioning but not in metabolism or cell cycle growth. Tim saw reproducible changes in yeast in the Rotating Wall Vessel (only 5 data points but could align promoters and get some candidate motifs).

Experiments that should be conducted in space with yeast

- Bud replacement. Yeast cells grow with stereotypical pattern relevant to previous bud cycle and is dependent on regulatory apparatus which directs cells where to polarize. This phenomenon of how cells orient themselves is highly conserved in many different organisms. So, any disruption in yeast cell polarity often results in that cell's ability to replace their bud sites. This is a sensitive assay to evaluate whether cells have undergone some perturbation. It is a well-defined pathway, most of the genes are known.
- Actin/cytoskeleton organization – very easy to do.
- Nuclear orientation – organization of mitotic spindle

- Organization of membrane structures ; vacuoles are good markers
- Chitin distribution – bud scars – indicates how well cells are processing in 3D space
- DNA content – measure with flow cytometry. Fix cells in 70% ethanol, pull off media. Will demonstrate which phase (stationary or log) cells are in.
- Mutagenesis – look at returned cells for mutations.
- Mitochondrial function – yeast is most powerful organism to evaluate mitochondrial function. It doesn't require respiration. Can evaluate cells to respire after treatment or perturbation – look at colony color or ability of cells to grow on non-fermentable carbon sources.
- Meiosis – there are yeast strains which go into meiosis when media is changed to a low nitrogen source. Any disruption in meiotic progress may reflect some disruptive effect from microgravity environment. Eric believes that microgravity will affect meiosis. The experiment involves initiating meiosis in culture and analyzing (1) the degree to which cells enter and complete the process (2) the frequency of non-disjunction (3) the inordinate frequency of mutant spores and (4) partial loss of chromosomes. An easy experiment.

Preservation of in-flight data

- Don't need fixation, spores are stable for weeks.
- Will probably see decrease in tetrads, will see diads.
- Grow in ideal medium, remove and resuspend in second medium. Have to get rid of all traces of media.
- BioServe hardware has the potential for adding additional solutions.

Re-entry effects

- Can be determined by (1) fixing or (2) going through 2-days of sporulation and comparing to yeast cells that have completed meiosis to determine if there are any further changes.

Initiation of experiment

- Can start either on the ground or in space.

Types of assays

- Deletion Project uses different array (universal tag array) specific for tags in deletion strains and measures only the tags. Determines the number of copies of the specific gene that has been deleted. Need to run time points for regular array as well. Difference between regular and deletion arrays is that deletion arrays tell you which genes are essential for growth; regular arrays tell which genes are expressed.

Diploids vs. Haploids

- Diploids are used for meiosis and Deletion theory. Haploids used for everything else. *Candida albicans* exhibits haploid growth, cannot go into meiosis, goes into a foraging behavior mode.

Fluorescent conjugates

- Can use to do translocation studies - different colors.

In-flight imaging

- CCD camera developed at KSC (flight qualified); resolution required for individual cell (10 microns across) to see how it responds as it is growing. Need only to look at presence or absence of fluorescence to measure kinetics of gene expression – one gene at a time. Gene expression, per se, is measured using arrays.

Deletion experiments

- Requires one vessel culture, all deletion strains, sample 6-7 times during flight, is compatible with every other experiment, chill down to 4°C (or can fix with formaldehyde), can start either on the ground or once in microgravity.

Gene Expression experiments

- Also can start on the ground or in-flight. Start with lyophilized cells and add media in flight. There are 3 types of yeast: *Saccharomyces cerevisiae* and wild type are useful for gene expression studies. *E. cerevisiae* (?) strain used to analyze effects of functional genes. In addition, *Candida albicans* can be compared to *Saccharomyces cerevisiae* in expression studies.

Other flight opportunities

- Stanford University has Picosatellite, a student program that is supported by several federal agencies and other universities. Hardware consists of picocubes (4x4x4, 4x4x8, or 4x4x12). Can fly groups of up to 9. There is opportunity to fly simple experiment in November. Next year they are thinking of doing a Lunar SlingShot – around the moon and back although the samples are not returned. Use modular transmission equipment, which transmits back to Rand Bldg. Stanford University. Cost \$50-\$100K. No biological payload has yet been flown and we have proposed a simple yeast survival experiment. Hydrating lyophilized yeast cells not easy. Can rehydrate yeast by immobilizing crystals onto filter. A yeast and plant experiment was proposed along with looking at features of culture growth with CCD camera.
- Space Explorers EPO program run by the state of Wisconsin also offers flight opportunities. This is an educational outreach program, which provided classrooms with the Near Earth Asteroid Rendezvous and Lunar Prospector events.

Hardware display

- It was suggested that BioServe be invited to Ames for further discussions of their hardware for potential flight with yeast.

WORKSHOP CONCLUSIONS AND RECOMMENDATIONS

The *Saccharomyces cerevisiae* Working Group concluded the following:

- **Brewer's yeast is one of the most important organisms 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 molecular mechanisms of response to the space environment 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 organisms in space may help us understand features of terrestrial life such as gravity that cannot be determined 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 may provide 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 mid-deck 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 mid-deck class accommodations. The major challenge is in preserving the in-flight 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. The complete set of results from the Yeast Deletion Project at Stanford University is available at the *Saccharomyces cerevisiae* Genome Database (SGD). 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 Stolc, 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 definitive.** 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 mid-deck 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 mid-deck 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.

Type of experiments recommended by the Working Group included:

- **Phenotypical profiles:** bud placement on fixed cells, organization of actin skeleton, nuclei and mitotic spindle, membrane structure and chitin distribution.
- **Cell cycle and DNA content:** determined by flow cytometry with fixed cells.
- **Mutagenesis:** evaluated by measuring respiration and, hence, mitochondrial function, by determining the ability to grow and by documenting the color of cells in culture.

- **Meiosis:** determined by studying nitrogen-starved cells that go into meiosis with media change or survival of spores.
- **Gene Expression profiles:** whole genome expressions of different strains should be examined in the same flight to give fast functional genomic analyses.
- **Gene Deletion Theory:** the use of mutants can demonstrate which genes are essential for adaptation to the space environment.
- **Gene Translocation:** using fluorescent tag (GFP), can determine transcription factors and specific pathways.

: Design concepts for yeast studies recommended by the Working Group:

- **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 albicans* 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 characteristics, organization of membrane structures, mitochondria, etc.
- **Gene expression, proteomics, gene regulatory mechanisms, and structure/metabolism should be examined together on a single flight.**

- **Cells should be spun down in-flight and fixed for archiving.**

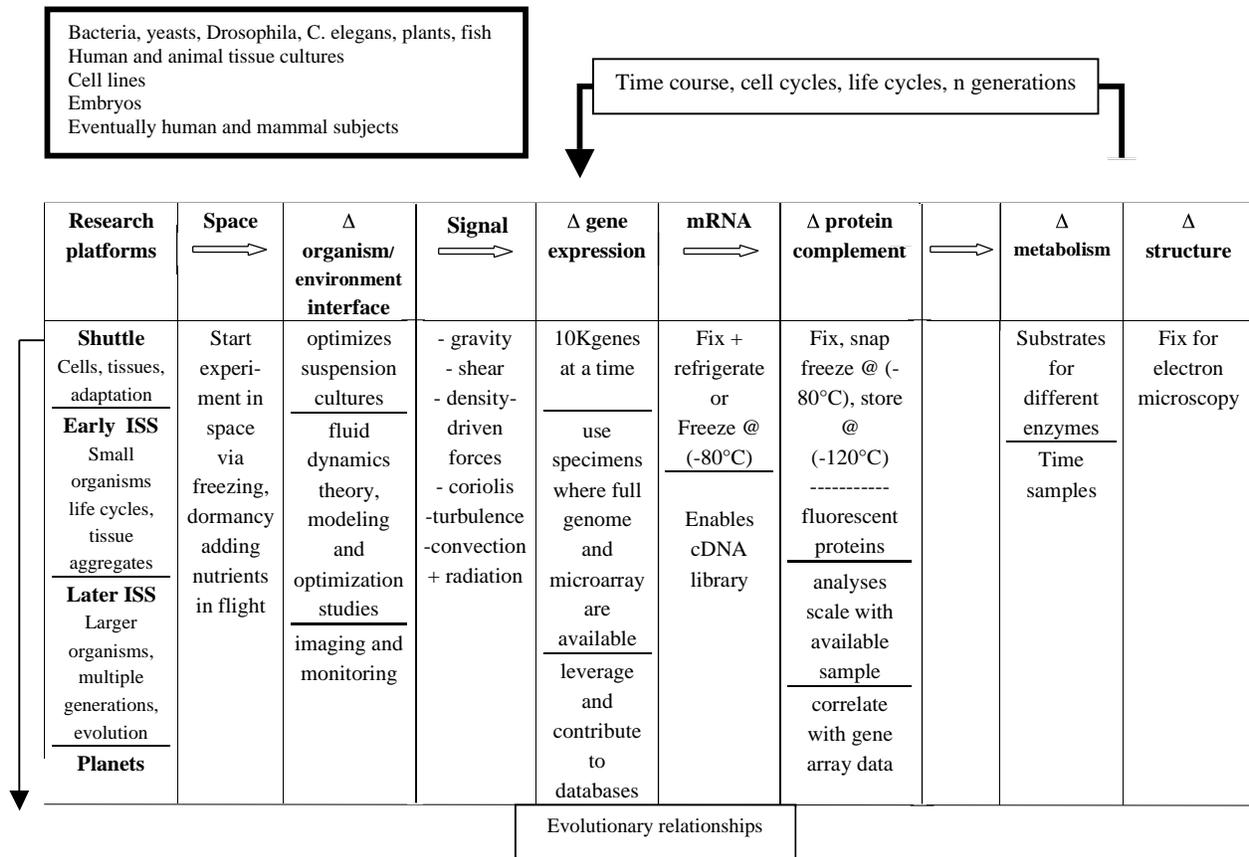
With the exception of the necessary confirmation flight, obtaining all of the data described above could be carried out in 1 and at the most 2 mid-deck lockers on a single flight. After this, the array of possible follow-up studies becomes so vast that either a workshop or an NRA is recommended to define 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 initial 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 in-house molecular biologists. The investigations can be carried out on commercial flight hardware and samples fixed in-flight. The samples can be analyzed 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.

The consensus of the Working Group was that yeast is an excellent model for what is going on in humans and should be a high priority species to fly in space. A pioneering yeast investigation could be ready for spaceflight implementation within 8 months using available flight opportunities and flight hardware. The results are expected to provide the fundamental basis for understanding animal cell biology, including human biology, in space.

Figure 1. Schematic of Molecular Biology of Life in Space



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 (not all inclusive)

http://web-x.arc.nasa.gov/ImageDB/experiment_images.cfm?ID=60

http://web-x.arc.nasa.gov/ImageDB/experiment_images.cfm?ID=145

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>

