Kibo Utilization Scenario toward 2020 in the field of Life Science

The Kibo Utilization Promotion Committee
The Scenario Examination Working Group in Life Science

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1. Introduction

Since life first appeared on Earth, the various processes of evolution have led it from the sea to the land and from the land to the sky. Humans, the inhabitants of Earth and benefactors of evolution, have been interested in space since their beginning, have studied in space, and have continued to fuel their passion about traveling in space. Through science and technology, we are now almost able to make the dream of ordinary people traveling to space come true.

Meanwhile, the International Space Station (ISS) program, which our country participates in and carries out under international cooperation, took nearly 30 years to complete after its initial planning phase, and full-scale operation and utilization have begun. The ISS is a symbol of international collaboration and a common legacy for mankind that develops not only up-to-date knowledge but also the spirit and experience of human beings.

Before we can extend our reach further into space, we must first comprehensively elucidate the biological response mechanisms that show the influences of the space environment on life, and how life responds to space. We must also understand the fundamental and physiological effects of human activity in space and long-duration missions on the living body.

This scenario includes an activity plan for life sciences research in the Japanese Experiment Module, Kibo, on the International Space Station, and it envisions development after 2020 aimed at coping with the basic proposition of life sciences in space. We hope that the knowledge gained by implementing this scenario will contribute to humanity's advancement in space and improve life on Earth.

1.1. Guidelines for the Scenario Study

A decision made by the Strategic Headquarters for Space Policy in August 2010 has led to Japan's continued participation in the ISS Program beyond 2016. A report prepared by the Expert Examination Committee for Space Policy in August 2011 shows that "Results in fields such as life sciences and observation have been obtained. However, it is necessary to expand upon the research through the characteristics of manned missions and to achieve concrete results from this point forward". While considering certain political situations, such as the financial situation of Japan and the fourth term of the Science and Technology Basic Plan, the president of JAXA commissioned the Kibo Utilization Promotion Committee, an external consultative body chaired by Dr. Makoto Asashima, to prepare Kibo utilization scenarios toward 2020.

Examination started in October 2010.

The Kibo Utilization Promotion Committee has provided the following approaches as indicators of prioritization of the Kibo utilization until 2020:

View point 1: Forefront scientific research only enabled by ISS/Kibo

1. Obtaining scientific knowledge in life sciences and material sciences over a long term (more than 5 years)
2. Creating such breakthrough technologies and making such findings in the short term (approximately 3 years) as those listed below:
    - Contributions to social problem solving, disaster recovery, etc., on the ground
    - Contributions to green/life innovation, creation of new industries, education, general use, etc.

View point 2: Fundamental research and development for the future space activities

Accumulating fundamental technology and knowledge in life sciences, space medicine, and technology development for Japan's lunar planetary exploration and manned expeditions

In addition to prioritized subjects, it is important to continue proposing excellent utilization subjects using a bottom-up approach and recruiting research themes by public announcement with providing some examples.

Based on discussions had by the Kibo Utilization Promotion Committee, a subordinate working group, the Scenario Examination Working Group, was established through the cooperation of life sciences specialists and the Space Environment Utilization Scientific Committee of the JAXA Institute of Space and Astronautical Science. The group will discuss approaches in life sciences including those mentioned above.

In keeping with the opinions of the space life sciences community, the working group created this scenario regarding the targets of ISS/Kibo utilization for life sciences research until 2020 and the experiment devices and promotion systems required to achieve those target, considering of future life science after 2020.

Moreover, research areas related to the health care and risk mitigation of astronauts must be promoted based on the space medicine research scenario. A comprehensive approach to life sciences as whole is necessary and it is important to promote the research in cooperation with other areas. Along these lines, this scenario in life sciences includes fundamental biomedicine research on humans, but does not include space medicine research contributing to the health care of astronauts.

2. Present Conditions and Trends in Space Life Sciences Research

2.1. Purpose of Life Sciences Research using the Space Environment

Life has adapted to various environments through fine mechanisms and has made great progress since its beginnings on Earth. All of this was accomplished under the force of Earth's gravity (1G). Now, it may be possible to discover effects or new abilities that have stayed hidden under Earth's gravity by studying the behavior of life in an environment different from Earth's. Additionally, the attempts to clarify the variety and complexity of these vital mechanisms are expected to lead to the investigation of a universal law of life that is not limited by the environmental conditions on Earth. This is one purpose of life sciences research using the space environment: to clarify the responsiveness and adaptive process of living organisms towards factors found in the space environment, such as microgravity, complex mixed radiation field, first at a micro level and later at a macro level.

Humankind has already advanced into space and the opportunities to survive long-term in an environment of microgravity and space radiation, where the conditions vary greatly from those on the ground, will be even greater in the future. To understand the fundamental and physiological effect of human activity and long-duration missions in space on living organisms, evaluating the influence of the space environment on living organisms, including humans, on genetic and cellular levels is essential. In addition, knowledge gained in space, such as space medicine research that finds countermeasures to muscle atrophy and bone loss in astronauts, should be used to care for and apply for the aging society on the ground. To this end, clarifying the mechanisms of and countermeasures to the effects of the space environment on humans through biomedicine is another purpose of life sciences research.

Life sciences research also covers the environmental control technology, life support, and food production techniques required for humans to live in space. And the origin and evolution of terrestrial and extraterrestrial life. It touches on the global desire to understand the Earth's environment, resources, and the occurrence of life on a global scale and is expected to improve in the future as an important research area. Life sciences research draws interest from the public because it satisfies intellectual curiosity and is related to daily life and health. It will also contribute to promoting space environment utilization.

2.2. Utilization Phases 1 and 2

Utilization is divided into three-year phases to recruit Kibo utilization themes and to establish a plan. The phases are as follows:

- Utilization Phase 1: from 2008 (Kibo utilization started) to the middle of 2010 (on the assumption of space shuttle retirement)
- Utilization Phase 2: from the middle of 2010 to 2012
- Utilization Phase 3: from 2013 to 2015

Utilization Phase 1 aimed to clarify the effect of the space environment at the cellular and molecular level, such as the expression of genes and proteins. This was Phase 1's priority subject and was based on "Japan's Future Approach for the Operation and Utilization of the International Space Station" (June 2004, Space Activities Commission). Utilization Phase 2 aimed to establish fundamental research on the aggregation of cells, targeting highly advanced life units (tissue, organs, individuals), and to clarify the adaptability of living organisms to the space environment. Preparation for an experiment using aquatic animals has started.

2.2.1. Prioritization Approaches and Research Items in Phase 1

The Space Environment Utilization Research Committee established a research scenario to indicate the future approach in life sciences in 2000. The scenario offered the two priorities described below as the research areas of life sciences in space along with future contributions to the Second-phase Science and Technology Basic Plan (in 2001).

- Elucidation of a signal transduction cascade, which is triggered by gravitational environment changes
- Integrated analysis of living organisms under the space environment over generations

  Meanwhile, after reviewing the ISS plan, the Investigative Commission of the Space Environment discussed prioritization of Japan's ISS/JEM utilization plan in 2001. Priority areas were determined in a roadmap with the future view of space environment utilization research in life sciences as described below:

- Phase 1 (from 2008 to the first half of 2010): Space Genomics

  Clarifying the effects of the space environment on living organisms at a cellular level, the smallest unit of life, and the altered expression of genes and proteins at the molecular level, and systematically verifying the ability of the space environment susceptive gene

- Phase 2 (from the latter half of 2010 to 2012) and Phase 3 (from 2013 to 2015): Space Behavioral Science

  Understanding the outcomes of Phase 1 systematically, and establishing and operating the fundamental research of a living organisms in a complicated unit (three-dimensional culture systems, physiological functions, neural networks, regenerative sciences, etc.), while aiming at progressing from a single cell level to research on multicellular system.

- Phase 4 and later (2016 and beyond): Space Environment Science

  Conducting research that clarifies the effects of the surrounding environment on a living organism and their general relationship

The revision of the roadmap created for Phase 1 is described in Fig. 1.

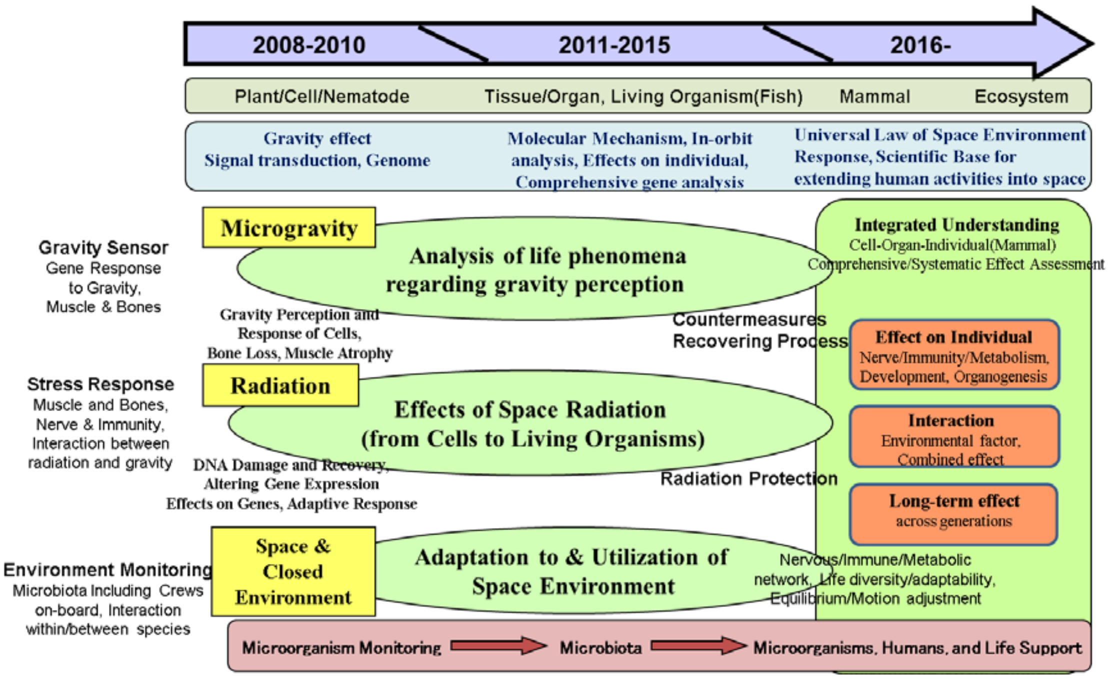

Fig.1 The roadmap of the space life sciences

・Research Items for Phase 1

Phase 1 aimed to obtain knowledge on cells, the smallest unit of life. The research was conducted under four themes as described below to verify the impacts, such as genetic changes and protein expressions, of the space environment (especially microgravity and radiation) at the cellular and molecular level.

(1) Cell biology

Analysis of a molecular group that vacillate its appearance under the microgravity environment, especially the genes and proteins related to muscle structure and breakdown, and research on the molecular mechanism in muscle atrophy under microgravity

(2) Neurophysiology

Analysis of the relationship between muscle atrophy and nerves, and the impact of microgravity on the vestibule to central nervous systems, using the altered expression and function of genes and proteins as indicators

(3) Radiation effect

Analysis of the impact of low-dose, long-term exposure to space radiation on living organisms at a genetic level, identification of the gene that responds to space radiation, and research on the interaction between microgravity and space radiation

(4) Plant Physiology

Analysis of the molecular mechanism of gravitropism and changes the strength of cell walls depending on the gravity change and the molecular structure of gravity reception machinery, and research on the impact of microgravity environment on the morphogenesis of higher plants.

2.2.2. Prioritization Approaches and Research Items in Phase 2

The targets, approaches, and research items for Utilization Phase 2 determined by the Kibo Utilization Promotion Committee in July 2007 are described below as:

- Accumulation of knowledge to establish countermeasures to expected problems in space life sciences, along with knowledge in environmental biology to establish a biological system, including living environmental maintenance and supply of food, in cooperation with space medicine
- In view of the evolution of life under the 1G environment, an aim to verify the variety and complexity of the behavior of life under an environment different from that on the ground, the mechanism of life on Earth, and to help understand comprehensively the life phenomenon, which is also a prioritized subject in life sciences on the ground

(a) Elucidation of the gravisensing and the anti-gravity response (verification of a gravity sensor and its network)

Verification of life's basic adaptation and response mechanisms to the space environment by gene and molecular levels based on the results from Phase 1

- Functional analysis of genes and protein complexes in the responses to space environment and changes in gravity
- Integrated understanding of molecular mechanisms from the reception mechanism of gravity change to the resulting response

(b) Elucidation of cellular and physiological function adjustment under the space environment, such as gravity (the basic biomedicine of space environment stress response)

Accumulation of scientific knowledge on medicine and biology, which is necessary to expand the area of human activities and to establish more effective countermeasures and solutions

- Elucidating the mechanisms of and developing countermeasures to muscle atrophy and bone loss
- Integrated understanding of the impact of space radiation on living organisms
- Elucidating the interaction between microgravity and space radiation
- Stress response of the brain and nervous system to microgravity

(c) Adaptation of living organisms to the space environment (the long-term influence of the space environment, space microbiology)

Clarifying the critical point of the alternation of generations in space and accumulating the basic data required for future long-term missions

- Consequence assessment of the latter term of organogenesis after its initial development using model vertebrates (small fish and amphibians) that can live for a long time in space
- Consequence assessment of reproductive cells across generations

Accumulation of the basic data required to maintain a sound environment of microorganisms inside the spacecraft and investigation of the immutable laws of life, which is not limited under Earth's environment

- Research on the relationship between microorganisms, and the relevance between microorganism and higher forms of life
- Possible microbial transformation (population dynamics) inside a living organism (the mouth or inside the intestines) caused by a long-term mission

(d) Initiatives for life exploration outside Earth and biological and ecological engineering (space utilization science)

Cultivating a better understanding of the origin of life, evolution, and distribution in space and research on life support technology using a closed ecological system

- Astrobiology
- Planetary biology
- Biological/ecological engineering

2.3. Status of Achievements and Outcomes of Kibo Utilization Experiments

An on-orbit experiment in life sciences aboard the ISS/Kibo (international public announcement selection theme, Kibo (JEM) utilization primary selection theme) started in October 2008. To date (January 2012), thirteen themes have been conducted: four in cellular organisms and neurophysiology, four in radiation effects, and five in plant physiology (including two themes, Cell Wall and Resist Wall, conducted by a European plant experiment device). Post-flight analysis and outcomes, as well as, research on plant and on microorganism are underway. The keywords and abbreviated titles of past themes are described according to year in Table 1.

Table 1 Keywords and Abbreviated Titles of Life Science Experiment Themes Implemented on the ISS/Kibo

Year	2008 (Utilizing other partner's equipment)	2009 (Life science experiments on Kibo start.)	2010	2011 (Retirement of the Space Shuttle)
Cellular Physiology & Neurophysiology		- Morphogenesis (DomeGene) - Nematode RNAi (CERISE)	- Muscle atrophy (MyoLab) - Scales (Fish scales)	
Radiation effects		- p53 Regulatory gene (RadGene) - TK mutants (LOH) - Silkworm (RadSilk)	- Nerve cells (NeuroRad)	
Plants	- Antigravity response (ResistWall) - Supporting tissue formation (CellWall)	- Higher plant life cycle (SpaceSeed)	- Hydrotropism (HydroTropi) - Cell Wall (Ferulate)	- Auxin dynamics (CsPINs)
Microorganisms		- Monitoring #1 (Microbe 1)		- Monitoring #2 (Microbe 2)

2.4. Challenges Recognized through Phases 1 and 2

Space experiments, especially on-orbit experiments in life sciences, face many more limitations than ground-based experiments do. Most discussions during the adjustment period of experimental design for Phases 1 and 2 of life sciences experiments focused on how to gain the best results under these constraints and the suitability of the experiment conditions. Past orbital experiments established the best experiment plans based on the constraints at the time. However, the subjects to be considered for the next phase through feedback to this scenario or the future policy for plan design are found as well. The main subjects are described as follows:

(1) Plan and Policy Formulation

- The scientific significance of utilization research themes in Phases 1 and 2 is recognized, yet the themes are not understood systematically as they pertain to space life sciences.
- Past experiments in scientific fields were selected individually through public announcements and were limited according to assumed schedules and available experiment devices. Therefore, implementation of many research tasks and corroborative experiments with multiple research tasks was not possible.

(2) Experiment and Research Environment

- Retirement of the space shuttle in 2011 led to the reduction of biological samples carried to space. This caused delays in implementation and required the review of research plans.
- Limitation of experiment resources in orbit (crew time, experiment devices, cold stowage, transportation capability) forces the amount of content need to carry out the experiment to be cut, and that leads to a lack of samples and data during the post-flight evaluation.
- Although mice and rats are effective model organisms, opportunities to conduct an experiment using small animals in Japan is extremely low and limited to sample sharing among researchers. If an experiment device for small animals cannot be used on its own initiative, research on individuals with the possibility of future research on humans will be restricted.
- The outcomes of past space experiments can be seen in scientific papers and progress reports. To analyze the general, cross-sectional mechanism that responds to the space environment, it is necessary to comprehensively compile a database for the information on genes and proteins that respond to the space environment.

However, a database with details of experimental results does not exist.

2.5. International Trends in Space Life Sciences Research

(1) The United States

In May 2009, the National Academy of Sciences (NAS) gathered proposals from the academic community on NASA's ten-year activity plan in space life and physical sciences by request of NASA and published them as a decadal survey in April 2011. The research subjects related to this scenario were as follows:

1) **Plant and Microbial Biology**

- Multigenerational studies of International Space Station microbial population dynamics;
- Plant and microbial growth and physiological responses; and
- Roles of microbial and plant systems in long-term life support systems.

2) **Animal and Human Biology**

- Studies of bone preservation and bone-loss reversibility factors and countermeasures, including pharmaceutical therapies;
- In-flight animal studies of bone loss and pharmaceutical countermeasures;
- T cell activation and mechanisms of immune system changes during spaceflight;
- Animal studies incorporating immunization challenges in space; and
- Studies of multigenerational functional and structural changes in rodents in space.

3) **Cross-Cutting Issues for Humans in the Space Environment**

- Integrative, multisystem mechanisms of post-landing orthostatic intolerance;
- Countermeasure testing of artificial gravity;
- Food, nutrition, and energy balance in astronauts;
- Continued studies of short- and long-term radiation effects in astronauts and animals; and
- Cell studies of radiation toxicity endpoints.

The report indicates priority-setting policy, risk and cost reduction of space exploration, research that can be done only by NASA, the synergistic effect with other agency's needs, and the necessity and effectiveness of space environment utilization. NASA has started a discussion to allow the use of animal experiment devices made for the space shuttle in the ISS based on these proposals.

(2) Europe

The European Space Agency (ESA) establishes "the Programme for European Life and Physical Sciences in Space (ELIPS)" every three years to promote the ISS utilization and ground-based research. Researches are currently underway based on the third phase of ELIPS (2008-2011).

Additionally, the European Science Foundation (ESF) has concluded the "Towards Human Exploration of Space: A European Strategy (THESEUS)", which is the European roadmap in life sciences research for human exploration using EU funding. THESEUS is presented to ESA after an examination by five groups of specialists in integrated physiology, psychology/human mechanical systems, space radiation, residence management (environmental microorganism, life support), and health management.

(3) Russia

The Russian Federal Space Agency (FSA) implements ISS experiments within the framework of the "Long-term Program of Scientific and Applied Experiments". Russia is the only country that has human transportation capability and supply return capacity from the ISS. The FSA carries out joint scientific research in the ISS with other countries, including the United States, Europe, and Japan, as an exchange of scientific results benefitting both countries. JAXA is currently implementing and discussing cooperation with Roscosmos in high-quality protein crystal growth research, space radiation environmental monitoring research, and research on aquatic animals.

(4) China

China is cooperating with space agencies in Germany and France to advance utilization of its flight opportunity, which includes China's Shenzhou spacecraft. Shenzhou 8, launched in November 2011, carried a joint experimental facility called SIMBOX (Science in Microgravity Box), developed with the German Aerospace Center (DLR). SIMBOX carried 17 experiments using plants, nematodes, bacteria, and human cancer cells along with a centrifugal incubator used to produce artificial gravity.

3. Future Approaches and Research for Space Life Sciences in Kibo

The following priority objectives until 2020 were established to promote space life sciences research in Kibo with consideration given to the outcomes of Kibo utilization in space life sciences to date, the approaches of Kibo utilization mentioned in Section 1, and the purposes of space life sciences mentioned in Section 2.1.

3.1. Prioritized Goal 1: "Integrative comprehension of adaptation process by living organisms to the space environment "

Sequencing the human genome was successfully completed in the early twenty-first century. Along with this epoch-making event, significant growth in the number and diversity of available genomes has dramatically accelerated ground-based discoveries in molecular biology. The successes of molecular biology have derived from the exploration of that once-unknown world by means of new technologies designed for analyzing and observing single molecules, as well as from increased availability and development of bioinformatics methods and computational technologies to determine the biological function and/or structure of the genes. Nowadays, most life sciences researchers use the same analytical techniques and technologies to understand the molecular and genetic mechanisms that are responsible for the functioning of the human body.

On the other hand, to elucidate the biological responses to gravity at the molecular level, experiments have been conducted in space conditions (microgravity), which indicates that the findings have important implications for manned spaceflight. Although these space experiments contribute to accumulating and expanding relevant knowledge and insight, further studies are required to elucidate systematically underlying molecular mechanisms as potential explanations for responses to an adverse environment. As an example, we have not yet determined the role of molecules involved in the mechanism by which animal cells sense gravity.

Exposure to space radiation involves serious DNA damage, mutation induction, and other potential biological effects, but further studies are required to gain multidisciplinary integrated understanding of the effects of space radiation.

As a result, detailed analysis using advanced relevant techniques is required to elucidate the biological effects of the space environment on molecular mechanisms. To do this, "multidisciplinary integrated approaches to understanding biological response mechanisms in the space environment," where the goals can be achieved only by utilization of the ISS/Kibo environment, will be pursued under View point 1, as described in Section 1.1.

The following research subjects are expected for this prioritized objective. Priority items for each research area are described in Section 4.

(1) Verification of the gravity response mechanism of living organisms and the gravity sensor

- Molecular imaging analysis of the movement inside a cell
- Functional analysis of gene and protein groups that respond to the space environment and gravity change
- Integrated understanding of molecular mechanisms at work from the reception of changes to gravity to the response
- Verification of the gravity sensor and its network

(2) Global analysis of the effects of the space environment

- Next-generation, comprehensive, integrated gene analysis
- Epigenetics

(3) Integrated understanding of the effects of space radiation on living organisms

- Verification of interaction between microgravity and space radiation

(4) Environmental adaptation and evolution of living organisms

- Horizontal transmission microbial gene
- Research on the relationship between microbes and between microbes and higher forms of life
- Changes in the microbe inside a living body (mouth or intestinal) caused by a long-term mission in space

(5) Comprehensive long-term evaluation of the effects of the space environment (influence on blood flow, nerves, hormones, metabolism)

- Integrated evaluation on the effects of the space environment using model vertebrates (Medaka, zebra fish, African clawed frogs, mice, and rats)
- Influence analysis of the latter term of organogenesis followed by initial development
- Influence analysis on reproductive cells across generations

3.2. Prioritized Goal 2: "Building-up scientific knowledge bases to expand human activity into space"

The Basic Space Plan (June 2009) and the Strategic Policy for the Space Sector (May 2010) published by the Strategic Headquarters for Space Policy propose "aiming to expand the area of human activities" and "aiming to establish the technical foundation for Japan's original future manned space activity." Humankind, including Japanese, is expected to continue performing long-term missions in space while the ISS is operational and thereafter.

JAXA is required as Japan's space agency to promote researches to understand and elucidate the basic physiological impact of human activity in space and long-term missions on a living organism.JAXA is also expected to systematically understand the effect of the space environment on cells and organs through research outcomes and to report these findings back to society.

As such, we promote the "Building-up scientific knowledge bases to expand human activity into space" as the fundamental research of future human space activity mentioned in Section 1.1 of View point 2.

A research subject for this prioritized objective is the expectation of human space activity to travel beyond the Earth's orbit. The following research subjects were established to aid in systematically understanding the effect of the space environment on a living organism, to better understand the utilization outcomes of Phases 1 and 2, and deduce how the outcomes affect humans, to establish a scientific foundation for safe and productive human activities in space, and to develop a life support system. Prioritized items for each research area are described in Section 4.

(1) Biomedicine focusing mainly on the space environment stress response

- Elucidation of bone loss and muscle atrophy and a method to control and recover from them
- Stress response to the space environment in the brain and in the nervous, vestibular, and immune systems

(2) The long-term effects of space radiation

- Measurement and evaluation technology of the influence of low doses of prolonged exposure
- A protection study based on the research outcomes of the effects of space radiation on living organisms

(3) Fundamental research on long-term missions in space

- Microbe environmental monitoring, management, and control
- Probiotics (application of useful microbes, such as a lactic acid bacterium)
- Evaluation of the long-term space environmental effects using mice and other vertebrates (across generations)

(4) Life support technology

- Effective plant production, photosynthetic ability, plant use, food production, etc.
- Closed system life support, etc.

4. Establishment of Prioritized Items in Research Areas

The targets of life sciences research on the ground vary from cell to tissue to individual and depend on the subject or approach. The same variances occur in space biomedicine research. Although constraints on space experiments exist, research at a cellular level as well as research using model organisms is necessary to create a variety of outcomes.

Plants, nematodes (the genome analysis of which is complete or underway [C. elegans]), and vertebrates, such as Medaka and zebra fish, have already been used in experiments. Mammals (small animals, such as mice and rats) are the candidates for future experiments. Cells are the most fundamental unit to provide basic knowledge of living organisms. Plants are valid model organisms to verify the gravity response mechanism because of their clear response to gravity and the simplicity they offer to space experiments. Nematodes will be used for gene control and muscle, nerve; vertebrates will be used in experiments on bone/muscle loss, the nervous and immune systems and as experimental disease models; and mice and rats will be used in the analysis of physiological phenomena, the musculoskeletal, immune, and nervous systems, and as experimental disease models.

Although these model organisms should be accommodated hierarchically based on the phenomenon and the priority objectives described in Section 3, this scenario specifies the prioritized items (targets until 2020) based on the model organisms in Fig.2 and the cross-sectional research area divisions. Figure 2 shows the priority items for each research area described below.

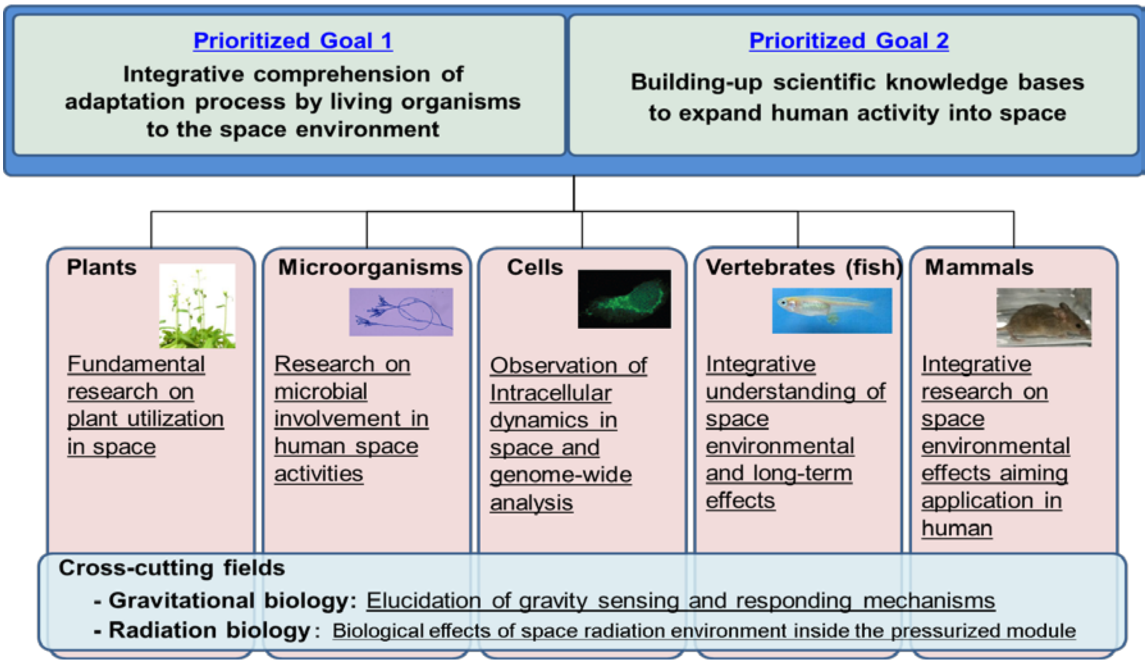

Fig.2 Prioritized Items based on the model organisms

4.1. Cross-sectional Research Area

Because the space environment, i.e., microgravity and space radiation, affects life in many ways, a cross-sectional approach to the field of biology is necessary to verify a universal mechanism that does not rely on living organisms. Additionally, a variety of unique subjects that Japan is expected to take the initiative in exist already in gravity response research or radiation effect research on living organisms using the space environment. It is important to promote more comprehensive and systematic research by gathering the knowledge gained from research using model organisms with clear genome information (nematodes or Medaka), mice and other small animals, and plants, and then developing further research utilizing their characteristics.

4.1.1. Gravitational Biology

・Prioritized Item: "Verification of gravity reception and response systems of living organisms"

Research on the gravity reception and response systems of living organisms under microgravity is the main research subject among all space life sciences research. Muscle cells cultivated in microgravity wither. Although the details are not understood, if the reception system, especially the acceptor, is found, it will be a great biological discovery and the first identification of a gravity sensor. It will also contribute not only to Priority Goal 1 but also to Prioritized Goal 2 by aiding in the development of countermeasures to bone loss and muscle atrophy in zero gravity. It is possible to add gravity stress to cells by activating the acceptor instead of exposing them to artificial gravity or a training machine. It is also expected to develop research results for elucidation of molecular foundation of gravity against gravity perception, countermeasure of bone loss and muscle atrophy, and measures to the aging.

Humans have adapted to and evolved in Earth's 1G environment and the origin and evolution of life on Earth are expected to be verified in space where gravity does not exist. Moreover, research in 1/6G, 1/3G, or partial G (phased gravity from 0-1G) and research related to the threshold value of the gravity reception and response of various model living organisms must be promoted in a cross-sectional and systematic approach as gravitational biology research for future lunar missions.

4.1.2. Radiobiology

・Prioritized Item: "Biological influence research of space radiation inside spacecraft"

The effect of long-term exposure to low-dose radiation on living organisms has been recognized as an important research theme, and individual phenomena, such as gene damage and the causes of mutation, have been studied. Yet this is not enough. Research that provides an integrated understanding is required.

Long-term genetic instability and epigenetic abnormalities are examples of the biological influence of low-dose radiation. To study the effects of radiation, using mammal cells, such as those of a mouse or individual, is desirable. However, if they are not available, then research using fish or amphibians will suffice.

It is important to set the safety margin for low-dose radiation exposure by accumulating and integrating research. It is hoped that by studying the effects of space radiation and microgravity, from early on at the molecular level to an individual level, through carcinogenesis, and later in the next generation, we may progress to space radiation protection research.

4.2. Research Areas Compatible with Model Organisms

The conceptual diagram of model organisms in space life sciences research is shown in Fig. 3. The prioritized item for each model organism and its setting are described below.

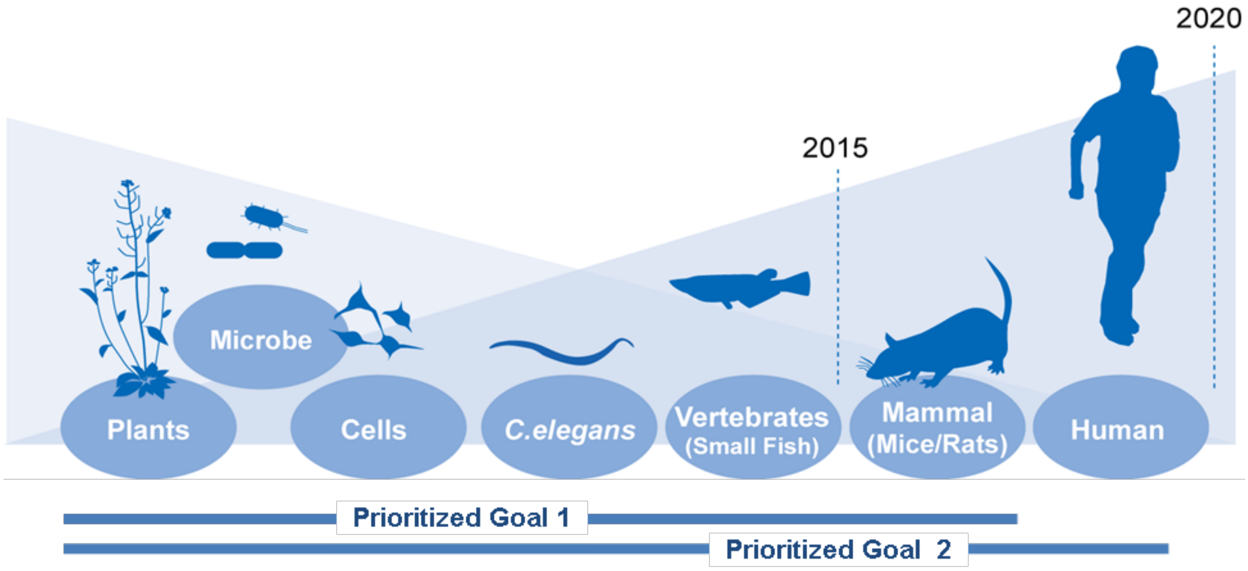

Fig.3 Conceptual diagram of model organisms

4.2.1. Plants

・Prioritized Item: "Fundamental research on plant utilization in space"

Simple space experiments as part of Prioritized Goal 1 (comprehension of adaptation process) involving cross-sectional research in gravitational biology and research in plant physiology will show a clear gravity response and will provide valuable information.

The priority items for plant research should be based on the fundamental and physiological research outcomes from the past, as well as future human flight. The aim should be to study the growth and differentiation of plants that are valid in enclosed spaces, physiological research, alternation of generations, and the fundamental research for development toward utilization phase. Examples of such research involve breeding higher plants in space, studying the photosynthetic ability utilization of plants in space (in cooperation with microbes related to cyanobacteria), verifying the capacity of plants that are capable of air filtration, and investigating fundamental gravity response plants for space agriculture.

Humans require reproducible food when staying in space long term. Consequently, research on the production, extraction, and refining of useful materials in space using plants will be priority subjects, as well as physiological research on the safety and integrity of plant breeding as food. In addition, technology used to produce plants efficiently must be established by verifying the process of plant growth in the space environment.

4.2.2. Microbes

・Prioritized Item: <u>"Research on microbial involvement in human space activities"</u>

The ISS is a completely closed environment and offers a very small amount of gravity. Therefore, it is a unique environment where microbes can fly and attach themselves to devices or humans (especially the exposed part of the body and head). Monitoring of microbes inside Kibo and the analysis of their movement must be continued to study their effect on humans and to prevent health hazards caused by microbes during a long-term space mission. In addition, an integrated monitoring and analysis system needs to be established in cooperation with NASA, ESA, and Russia not only for inside Kibo but also inside the entire ISS.

Research on the relationship between humans and microbes will be promoted as space microbiology in the future and will be based on human activity in space. Examples of research subjects are evaluation of integral effects of microgravity and space radiation, research on the propagation of cell genes as microbial ecosystems, space immunology research aimed at maintaining healthy life in space, research on opportunistic infections originating in the herpes virus or stress, and research on viruses related to fatigue.

One promising area of research is in probiotics, which actively uses microbes in the space environment. Metagenomics research of enteric bacterial flora from a lactic fermenting beverage or food intake is expected to contribute to the maintenance of the astronauts' health. Moreover, biostimulation research considering the application of producing food using excrement in space and prevention of immune system depression will be necessary in the future.

Relaxation during a long-term mission, air cleaning technology, production capacity, measurement of photosynthetic ability using cyanobacterium, a comparison experiment of carbon dioxide absorption and the oxygen evolution effect on the ground, and development of fundamental technology for a new enclosed life maintenance system are examples of application and improvement targets.

4.2.3. Cellular and Multicellular Organisms

・Prioritized Item: <u>"Observation of intracellular dynamics in space and genome-wide analysis"</u>

Since space experiments are full of constraints, experimentation using animal and plant cells is carried out as the core of space life sciences research for its efficiency. Experiments using cellular tissue of mammals, amphibians, and nematodes are essential to the advancement of research on gravitational biology or radiobiology and to detailed analyses of genes.

As a unique priority item of cell research, "observation of intracellular dynamics in space and genome-wide analysis" will introduce a molecular biological experimental method that is showing remarkable improvement on the ground and synergistic development of ground-based research and space experiments. Examples are the technology to observe and analyze the real-time movement changes of animal and plant cells' organelles under the space environment or implementation of various analytical methods in orbit instead of on the ground. Especially, integrated, comprehensive genome analysis (including epigenetics and epigenome) is necessary in the future to promote space environment utilization for molecular biology research, as described in Prioritized Goal 1. The key is to establish efficient analytical techniques and to integrate the information gained from each space experiment with all the model organisms. Moreover, interaction between cells or the combined effect with space environment factors (microgravity and radiation), verification of adaptation and recovery mechanisms towards the stress of space, and a common understanding of the effects of the space environment on cells to individuals are required.

4.2.4. Vertebrates

・Prioritized Item: "Integrative understanding of space environment and long-term effects"

Aquatic animals, such as small fish, cynops pyrrhogaster, and African clawed frogs, have been used for space experiments as individual model organisms because they are vertebrate like humans. Japan launched the Aquatic Habitat (AQH) and made possible long-term experiments using small fish (Medaka and zebra fish) inside Kibo. Among these aquatic animals, Medaka has been characterized in ground-based research utilizing various transgenic animals and mutant strains, with it's genome analyzed. In addition, Medaka, both the developing embryos and the adults, are suitable subjects for observation and visual recognition. Visualization of the active state of a particular cell and gene inside a live Medaka can be done using a fluorescence microscope. Since the generation time for Medaka and zebra fish is short compared to that of other vertebrates, breeding three generations is possible using the AQH. Fertilization and generation of Medaka has been carried out successfully in orbit (the IML-2 Space Shuttle mission).

Because of their characteristics, aquatic animals are effective model organisms to promote both prioritized goals. Examples of research subjects and the outcomes expected for Prioritized Goal 1 are as follows: verification of the response mechanism in vivo and at the molecular level related to bone metabolism and muscle atrophy using transgenic strains of Medaka and zebra fish; verification of the effect of radiation on reproductive cells and tissue; verification of the effect of gravity on the initial generation process or vestibular system; and influence evaluation of the space environment response mechanism across generations.

A long-term, quantitative evaluation of the effects of the space environment could be done through an experiment using aquatic animals for Prioritized Goal 2. Examples of research are evaluation of the effect of long-term exposure to low-dose radiation on the reproductive system (tissue and genes) progeny using mutants with various radiation sensitivity, evaluation of the effect of space environment on circulation, digestion, sensation (vestibular functions and reflections), and immune function, to various stresses (posture control or behavior pattern), as well as the process of recovery from those effects on the ground. Moreover, knowledge is expected to be gained regarding the differences in bone loss and muscle atrophy mechanisms between humans and model organisms in space.

1) Targets and Steps until 2015

- To implement the launch of AQH, scheduled in 2012, and initial on-orbit technological demonstration, Phase 2 utilization selection themes on bone metabolism, and experiments based on international cooperation, and to discuss and prepare the selected experiment candidate themes
- To gain the knowledge needed to evaluate the prolonged space environment effect on multiple generations, breeding Medaka or zebra fish for three generations in orbit using the AQH and their unique characteristic of a short generation time

2) Targets and Steps until 2020

- To integrate the outcomes of research on the multi-generation breeding experiments using the AQH or research focused on particular organs or tissue and to understand comprehensively the effect of the space environment at cellular, molecular, genetic levels to individual organisms. Especially, to use the research outcomes until 2015 to establish and promote priority research on the long-term effect of the space environment over generations.

  The AQH is a unique ISS experiment device that uses individual organisms and utilizes international cooperation to understand individual organisms systematically and deductively with knowledge gained at a cellular level, especially live phenomena.

4.2.5. Mammals

・Prioritized Item: "Integrative research on space environment effects using mammals (small animals) aiming application in human"

The biggest benefit of using mammals (small animals such as mice) as model organisms is that they are easier to adapt or relate to humans than other model organisms. It is also beneficial that more samples can be obtained at once since each individual is bigger than other model organisms used in space experiments. In addition, various transgenic and disease model in mice are available as experiment samples on the ground. Research outcomes that have used mice have had a large impact. Therefore, dramatic improvements in the experiment environment in orbit, such as increases in the participation of new and front-line researchers and a greater diversity of research outcomes are expected. Yet space experiments using mice still have many constraints. Integrated research with other model organisms, cellular research, and a projective approach to enable one experiment efficiently are necessary.

Those characteristics prove that small animals are effective model organisms for Prioritized Goal 1 and 2 (Fig. 3). Examples of research subjects for Prioritized Goal 1 are verification of the mammals' adaptation response mechanisms to the space environment, including bone metabolism and muscle atrophy mechanisms, multiple influence evaluation of immunity, protection, and metabolism, and explication of the space environment response mechanism of high level biological functions, such as those in the brain and nervous system.

Experiments using small animals is essential for establishing various scientific foundations that contribute to Prioritized Goal 2 and the expansion of long-term human activities in space. Examples are the evaluation of not only the prolonged effect of the space environment but also the recovery process, confirmation of each medicine's effect using diseases models, and influence evaluation on the whole system of a living organism, including metabolism and the immune system. Moreover, progressive research involving the reproduction of mammals, an influence evaluation on the initial development process, and modeling hibernators that are tolerant to environmental stress for a long period is expected.

1) Targets and Steps until 2015

- To establish missions for small animal experiments that Japan can conduct in cooperation with the research community and international partners and to implement the necessary fundamental research development, preliminary study , and international cooperation

2) Targets and Steps until 2020

- To establish an environment where Japan can implement a small animal experiment of its own initiative in the ISS and to promote experiments based on Prioritized Goals 1 and 2

4.3. Prioritization and Focus

Research subjects corresponding to the prioritized goals and based on Japan's strategy and policy, such as the support for reconstruction from disasters or growth strategy, should be promoted preferentially. Subjects that create technology and knowledge which make an breakthrough in a short period are also important. Examples of such research subject are as follows:

(1) Solutions to social problems on the ground and contribution to disaster recovery
(2) Contributions to life and green innovation
(3) Subjects that have a large effect on other areas, such as space medicine research and human technology development, and that need to be done in cooperation with other parties
(4) Subjects that analyze Prioritized Goals 1 and 2, mentioned above, in an integrated manner and fundamental technology development research

The following research subjects are expected for the items above:

- Elucidation of the influence of low doses of prolonged exposure to space radiation
- Elucidation of the muscle atrophy mechanism and control method which contributes to aging society
- Evaluation of the space environment stress response
- Evaluation of integrative and long-term effects of the space environment using model vertebrates (Medaka, zebrafish, mice, rats, etc.)
- Application of real-time monitoring technology for microbes, radiation, etc.

4.4. Expected Area of Future Prioritization

(1) Life Exploration outside Earth

It is suggested that liquid water flowed on the surface of Mars and the Moon, and there is a possibility that life once existed on Mars. Research to verify those hypotheses will clarify the origin of life and the limitation of life activity under extreme situations; provide the importance of Mars exploration; and evoke the national curiosity and interest in space. It is important for researchers in space life sciences to participate actively in a project to identify the existence of life outside Earth in cooperation with science and technology.

(2) Life Support Technology as Life Sciences Research

Integrated research on life support under the space environment (food, energy, medical treatment, environment, mini-ecosystem, etc.) as an application field of space life sciences research should be performed based on past research on plants, microbes, and cells.

5. Preparation of the Experiment Environment and the Basic Technology to Prioritization of Space Life Sciences Research

5.1. Basic Policy

Kibo is equipped with devices that assist in common life sciences experiments: the Cell Biology Experiment Facility (CBEF), Clean bench (CB), and the Minus Eighty-Degree Laboratory Freezer for ISS (MELFI). Although the Aquatic Habitat (AQH) and a fluorescence microscope are scheduled to be installed, new experiment devices are still needed to accomplish the prioritized goals and items mentioned in Sections 3 and 4. The discussion should be carried out by JAXA in cooperation with the Kibo Utilization Promotion Committee and community under the policy described below:

(1) Existing experiment devices and those under consideration must be used fully to accomplish the prioritized goals and items under the constraints of extremely limited resources.

(2) Although some experiments require devices owned by other countries, JAXA must consider their use and promote cooperation between space agencies.

(3) Moreover, an order of priority for the essential devices needed to accomplish the prioritized goals and items in Section 3 and 4 must be established and its preparation must be discussed with consideration given to Japan's superior research areas and the possibility that Japan can lead the international cooperation.

(4) Analytical instruments and observation devices for life sciences research have been remarkably developed on the ground. Installation of consumer products on the ISS will be considered actively based on those research and technology development trends on the ground.

(5) Data processing and acquisition in orbit, automation and miniaturization of devices, and minimization of recalls should be considered according to the limitations on on-orbit life sciences experiments (up/down mass, on-orbit cold stowage, reduction of crew time, etc.).

5.2. Preparation of the Experiment Environment to Achieve the Prioritized Goals and Items

5.2.1. On-orbit Experiment Environment

The following experiment devices and environment should be discussed to accomplish the prioritized goals and items mentioned in Sections 3 and 4, based on the policy described above.

(1) An on-orbit breeding and experiment device for small animals (mice, rats, etc.)
- It is essential to use small animals for experiments as model organisms to improve research progressing from cellular levels to that of humans, to link with the large and cutting-edge ground-based life sciences research outcomes, and to create top-level scientific outcomes.

(2) A molecular imaging instrument that can observe the dynamic changes inside cells
- Confocal microscope, reflecting microscope (molecular imaging technology), etc.

(3) A real-time, on-site microbe monitoring instrument
- Lab-on-a-chip technology, PCR, etc.

(4) An analytical instrument for comprehensive gene expression/epigenome

(5) A plant culture control and analysis instrument

(6) The function to launch and return living organisms
- Launch the living samples by Japan's H-II Transfer Vehicle and add the return function

(7) Organism experiments using external experiment platforms (ozone layer or the ultraviolet effect)

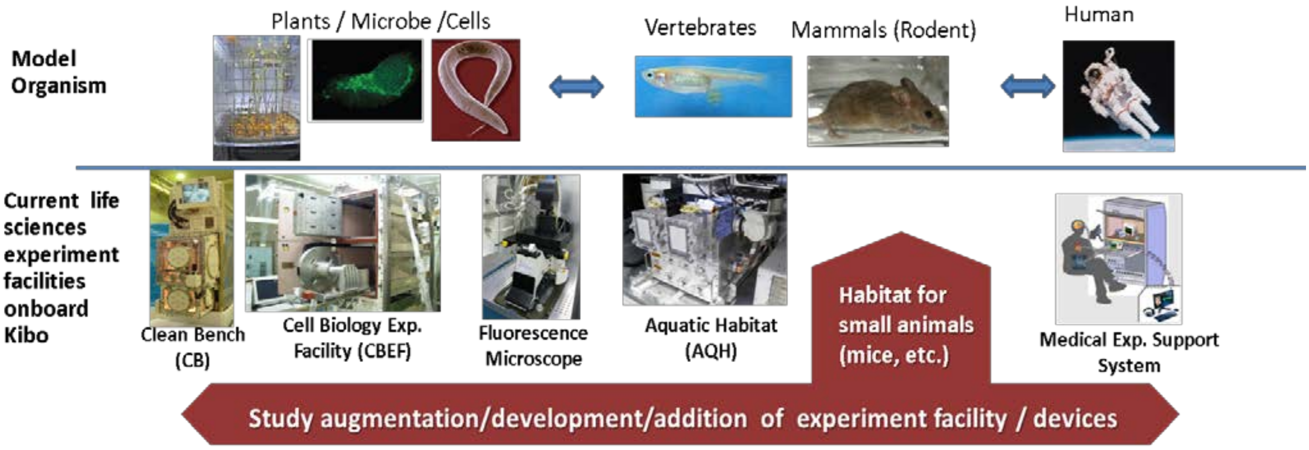

Fig.4 Kibo Life science facility/devices

5.2.2. Ground-based Experiment Support Environment

An environment where sufficient examinations and preliminary experiments on the ground can be performed is required to utilize the limited experiment opportunities in space and obtain the greatest possible outcomes. Since the number of experiments using Kibo has increased, it is necessary to discuss the system to efficiently accumulate more detailed experiment data. The following conditions are expected to support ground-based experiments:

(1) Comprehensive gene analyses using a next-generation sequencer for space experiment return samples

Although space experiment results, especially return samples, are extremely valuable, they are currently used only for individual analysis by a researcher (proposer). A discussion for universal/cross-sectional analysis of the response mechanism to the space environment will be made to analyze the comprehensive genome using a next-generation sequencer and to compile the database comprehensively (Fig. 5).

(2) Establishment of a database for gene expression (summarize and share the individual experiment outcomes)

The results of space experiments and the raw data and results of analysis using a next-generation sequencer will be compiled in a database comprehensively.

(3) Preparation of ground-based control experiment facilities

To establish the new on-orbit experiment devices described in Section 5.2.1, the setting policy for ground-based control groups and the experiment environment on the ground must be discussed.

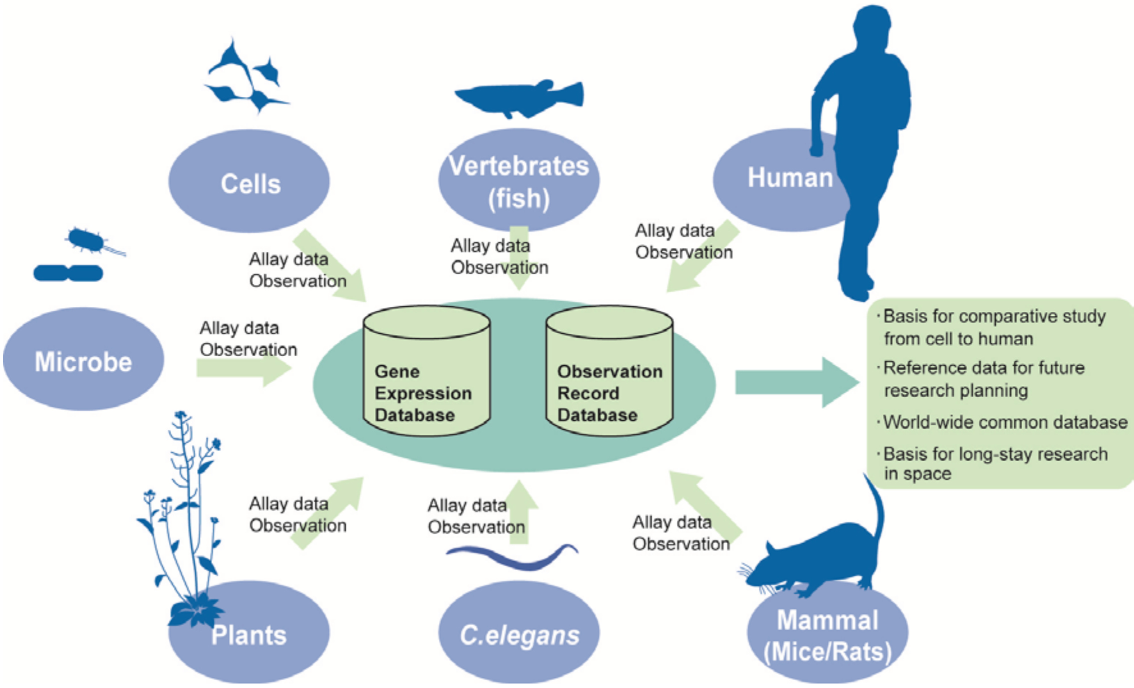

Fig.5 The space Life Sciences database priparation

6. Promotion Methods

The Life Sciences Scenario Working Group established the prioritized areas for life sciences in space environment utilization, as mentioned earlier. Moreover, the following items must be considered and conducted to promote the priorities effectively and obtain excellent research outcomes.

6.1. Basic Policy

(1) To solve the prioritized subjects by gathering the power of the research community and bottom-up opinions. To promote utilization in cooperation with the community.

(2) To expand the view of space environment utilization research and raise the level with participation of not only the limited user community in space related areas but also life sciences front-line researchers in molecular biology and medicine.

(3) To promote space life sciences research at JAXA by integrating JAXA's Human Space Systems and Utilization Mission Directorate, Space Environment Utilization Center, which performs apparatus development, experiment operation, and resource management of Kibo, and other divisions related to life sciences.

6.2. Selection of Prioritized Subjects

Although the working group proposed priority areas in life sciences while considering the current research trend and future development, they have not specified the prioritized subjects yet. This is because they thought in order to establish prioritized subjects for each area, it is necessary to set a project under the appropriate research representative and select subjects that are promoted by the executing research organizations. The selection of prioritized subjects should be made by the Kibo Utilization Promotion Committee with consideration given to prioritized areas proposed in this scenario and proposals through announcement of opportunity process. It is an appropriate process because it defines the targets and objectives of project and clarifies where responsibility lies with the principal researcher and his/her research institution.

6.3. Consideration for Selection of Prioritized Subjects

(1) To select subjects from the proposals of a public announcement, the originality and excellence of targets and the validity of the research system, research period, research plan, and research expenses must be evaluated.

(2) In addition, the research institution must have the capacity to continue the research not only for the project period but also for the duration of scientific and technical evaluation of the research results.

(3) The researches for this priority subject utilize the world's only ISS, and the selection and evaluation must have a global point of view. Priority subject research in the ISS must meet the international standard and the activity should be reported both domestically and globally, consideration of the opinions and evaluations of foreign researchers must be made in the selection of subjects. An example would be a paper screening or evaluation by instructive researchers in the West.

6.4. Evaluation System

Selected prioritized subjects and projects will be evaluated regularly by an exterior committee to check the progress of targets, evaluate the research outcomes, and conduct follow-ups effectively, including the review of the implementation plan and cancellation measures.

6.5. Consideration for Experiment Support Technology and Research Project Teams

(1) Experiment devices to be installed should not be limited to existing (or approved) generic facilities. Installation of the most appropriate device for the project must be discussed. To achieve excellent and unique research outcomes, an original experiment device is often required and development of such device should not be limited as long as the technical problems can be solved.
(2) Establishment of a research cooperation system in JAXA is necessary to implement the projects and prioritized subjects. A member of JAXA who has performed a space experiment needs to be part of the research team since the project requires development of experiment devices and project management. Existing devices have on occasion performed at a lower level than originally planned or failed during the experiment period. Therefore, high reliability for devices is expected. Close partnership should be built between JAXA's loading technical team and the research team, and the selection of prioritized subjects should be evaluated on their relevance to the partnership research promotion system.
(3) ISS experiments should involve the crews as little as possible.
(4) The ISS is one and only global joint research facility. Utilization should be done under the international evaluation standard and each research project should be evaluated globally. At the same time, an effort to establish international cooperation and a joint research system should be made.
(5) The data obtained from space experiments are extremely valuable and should be provided widely to the research community to share the data actively after a period of preferential utilization by the research project team.

6.6. Promotion of Ground-based Research

Promotion of ground-based researchers is expected, using the Grants-in-aid for Scientific Research (Space life sciences) established in 2012 with a time limit. In addition, selection of fight candidate themes in association with the Grants-in-aid for Scientific Research should be discussed

6.7. Cooperation with International Agencies

To utilize the limited space experiment opportunities to the greatest extent possible, active cooperation, such as an international life sciences meeting, joint use of experiment devices, integration of space experiments, and sharing of data, with international agencies must be discussed.

6.8. Outreach, Enhancement of Understanding, and Human Resource Development

The ISS program is a big project that requires large funding, so understanding and cooperation of general public are required for its promotion. The results of ISS and Kibo utilization and their propagation must be provided actively to the public in a wide-reaching and simple way. Research outcomes in life sciences, especially, are expected to get the attention of the nation due to their relation with the life and health of society and should be promoted actively.

Moreover, the experiment proposers (researchers) should summarize the professional and academic outcomes in a way that is easy to understand. Those documents will be used for education and to recruit future researchers in life sciences and participants in space experiments.

6.9. Research Areas to develop, using the Bottom-Up Method with Public Announcements

In addition to the promotion of prioritized subjects, it is important to continue encouraging unique and leading subject proposals from researcher using the bottom-up approach.

We hope to receive proposals in scientifically and technically promising areas that have no history of flight experiment and not yet to be JAXA prioritized subjects, in Grant-in-Aid for Exploratory Research or immature subjects, and in areas that is expecting to obtain new knowledge with Kibo (experiment devices).