

Programmable Plants: Development of an *in planta* System for the Remote Monitoring and Control of Plant Function for Life Support

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Abstract

In this Phase I study we explored the revolutionary concept of using genomics and nanotechnology together for the development of plants with traits suitable for human life support in non-Earth environments. This includes the potential for the use of remote monitoring and control devices *in planta* and *ex planta* as part of an overall architecture of human and autonomous control systems. This project explored concepts that could advance NASA's goal of solar system exploration and aligns with four of the five NASA Enterprises. With higher plants playing a central role, this system would provide the air, water, food, fiber, pharmaceuticals, chemical feedstock, and other materials needed to sustain human existence beyond Earth and ensure the success of the mission. The plants would work in concert with the human element (whether the humans were on Earth, in transit and on other planetary surfaces) and in concert with the support infrastructure such as greenhouses, harvesting/processing hardware and monitoring/control systems. Development and integration of a fully biologically based life support system that is genetically engineered and remotely controlled to act in response to the changing needs of the crew/team would be of enormous significance in the quest to establish outposts beyond Earth. The marriage and application of classical selection/breeding with the emergent fields of functional genomics and nanotechnology would bring NASA's goal of establishing a human presence on Mars closer to reality. In spite of the immense potential of programmable plants, a great deal of advanced concept planning, system development and basic research remains to be done. This report presents a plan and timetable for defining specific strategies to revolutionize our concept of bioregenerative life support for human solar system exploration.

Introduction and Rationale for the Study

Mankind will explore the solar system. In addition to being an innate desire of mankind to probe the unknown, this goal is part of the NASA Strategic Plan (NASA 1998). Exploration of the solar system is a specific goal of four of the five NASA Strategic Enterprises. There have been numerous plans to justify the reasons and map out strategies for this endeavor (Mars Reference Mission 1997, Stafford 1991, Zubrin 1989 and 1996).

To date, most of the focus has been on developing engineering and infrastructure technologies and systems. This has resulted in important advances in transport and propulsion systems (reviewed by Beardsley 1999). While these are crucial issues, there are other equally compelling considerations. As stated by Freeman Dyson "The chief problem for a manned mission is not getting there but learning how to survive after arrival. Surviving and making a home away from Earth are problems of biology rather than engineering." He goes on to state that "Any affordable program of manned exploration must be centered in biology". Finally he points out that "To make human space travel cheap, we will need advanced biotechnology in addition to advanced propulsion systems" (Dyson 1997). In the long term, success will depend on the ability of the inhabitants to live (and thrive) off the land and their ability to adapt to the new conditions in which they find themselves. In order for this to happen, technologies must be developed that make solar system exploration a great deal more dependable, economical, and sustainable.

The potential for achieving all three – using biology to survive, living (and thriving) off the land, and making the economics more favorable – can be attained by utilizing recent advances in the fields of genomics and nanotechnology. In a broad sense, genomics is the determination of entire genetic sequence of organisms, interpreting the vast amounts of information found within these sequences (bioinformatics), determining the function of large numbers of gene products (functional genomics), and manipulating the organism to achieve a desired end result (genetic engineering). There have been substantial advances in this field with microbes and animals, including humans (see *Science* vol. 282, 1998 and *Science* vol. 287, 2000) and with plants (see *Current Opinion in Plant Biology*, vol. 3, 2000).

Nanotechnology encompasses the development of tools and devices for the probing and manipulation of matter at nanometer scale dimensions. For example, advances in the ultraminiaturization of devices for microscopy (scanning probe microscope, scanning tunneling microscope, and atomic force microscope) have been used for this purpose (Mirkin 1999). The development of nanotube nanotweezers that can manipulate individual sub-micron-sized objects and directly probe their electrical properties has recently been demonstrated

(Kim and Lieber 1999). Additionally, conjugated polymer actuators have been made that operate in aqueous solutions (Jager et al. 2000) and nanoprocessing of soft materials has taken place, which would be more suitable than silicon- or glass-based structures for biological applications (Quake and Scherer, 2000). With these developments, the potential for developing intrinsic nanodevices for the control of biological function is nearer at hand.

Current life support technologies are entirely physico-chemical and are part of the "expendable resupply" philosophy that has served the US Space program well for the last several decades. At present, a physico-chemical life support system can provide clean air and water but cannot produce food. As mission duration and distance increase, the economics of a solely physico-chemical system becomes less favorable and the economics of a bioregenerative system become more favorable (Olsen 1982), primarily because the need to have a continuous resupply of food is removed. Since the production of food by plants comes as a consequence of photosynthesis (a process that uses light energy to remove and reduce CO_2 as well as produce O_2) and evapotranspiration (a process that purifies water), it makes sense to use plants for the total life support machine that they are.

Development and integration of a biologically based life support system that is designed to act in response to the changing needs of the crew/team and quickly adapt to its environment would be of enormous significance. The major component of this system will, of necessity, be plants. Plants have numerous built-in redundant systems (e.g., fail safe mechanisms for protein translation and DNA replication) that can be exploited to maximize success. Plants are solar powered and naturally self-perpetuating, obviating the need for expensive and potentially unreliable re-supply missions. An added and very important feature of plants is that their seeds are small, easy to transport, tolerant of extreme environments, long-lasting and store all the information needed for the subsequent plant to go through its entire life cycle.

Precise monitoring and control of all aspects of the human/plant/environment biosphere must be basic elements of the overall system. Because any program of solar system exploration will involve significant periods with humans not present or unavailable to spend time on plant culture, and because the stakes are so high (i.e. the success of the life support system), the integration of remotely controlled and smart nanotechnology to monitor and control the function and metabolism of plants will be a key and revolutionary development. In addition to enabling Mars habitation, this marriage of technology and science will have profound impacts on Earth agriculture and the efficient feeding of the rapidly expanding (Earth-bound) human population. Plants developed to survive and thrive the abiotic stresses of non-terrestrial environments will contribute significantly to the second green revolution (Conway and Toenniessen 1999). For these reasons, the development of plants specifically tailored for the provision of life support is not just beneficial, but becomes essential as we look to

permanently inhabit other bodies in our solar system and to serve as responsible stewards of our own.

We envisage a scenario where the selected plant species could be engineered and remotely controlled to provide clean air, potable water, and food while at the same time acting as a pharmaceutical factory and source of raw materials. Specifically engineered and controlled species could be integrated into a cohesive system to provide for immediate life support needs (air, water, food) while also supplying the necessities and enhancers for long-term presence (clothing, shelter, pharmaceuticals, materials, fiber, flavoring, perfumes, soaps). In addition to providing life support requirements, plants can be critical for the maintenance of the psychological well being of a crew, particularly on long-term missions or permanent settlements.

The work supported by this NIAC Phase I grant was focused on exploring the viability and defining major feasibility issues related to the development of a fully-automated, programmable, remotely-controlled bioregenerative life support system for Mars. The following components were considered and explored:

- The Martian environment with respect to biological system survival;
- Development and integration of nanoscale biosensors and control devices for use in and around the plants and plant-growing systems;
- Genetic engineering and/or selection of plants with traits suitable for surviving, thriving and providing for all human life support needs in Martian outposts or colonies;
- Development of a system of *in planta*, heritable nanodevices for the remote monitoring and control of biological function.

In this report we discuss each of these components with respect to the current state of the field and the realistic possibilities for progress. Overall, we determined that the development and integration of a biologically based life support system that is genetically engineered and remotely controlled to act in response to the changing needs of the Martian crew would not only be of enormous significance, but in fact is an absolute necessity for any consideration of long-term or permanent presence on Mars. With the current progress in plant functional genomics (Meinke and Tanksley 2000), this is a feasible option in the 10 to 40 year time frame. The need for a system of smart biosensors is also of critical importance in the functioning of such a life support system. Again, with the current and rapid progress in the areas of sensor technology, materials development and miniaturization (Service 2000), this too is feasible within 10 to 40 year time frame. The integration and application of functional genomics and nanotechnology towards a biologically based life support system would bring NASA's goal of establishing a human presence on Mars closer to reality.

However, a massive amount of advanced concept planning, along with systems development and basic research remains to be done.

The Martian Environment With Respect to Biology

What are the environmental conditions on Mars? While harsh for terrestrially adapted species, the conditions are not impossibly severe.

Light. Mars is 1.524 astronomical units from the Sun with a mean solar flux of 0.43 times that of Earth (Carr 1996). Martian day length is 24 hours and 39 minutes, with similar seasonal variations as on Earth. While dust storms occur, the amount of incident radiation during such storms is calculated at 15% of the clear sky value (Meyer and McKay 1989). Mars is virtually cloud free. In comparison, most agricultural areas on Earth have significant cloud cover such that the integrated solar energy reaching the surface of Mars may be at least equivalent to many productive areas on Earth (Salisbury 2000).

Atmosphere. The atmosphere of Mars is thin, with a mean pressure of 6.9-9.0 mbar or less than 1% of the pressure on Earth at sea level. It is made up primarily of CO₂ (95.3%) and also contains N₂ (2.7%), Ar (1.6%), O₂ (0.13%) and H₂O (0.03%) with other trace gasses present (Carr 1996).

Temperature. Martian surface temperature depends on the latitude, season and surface albedo, and ranges from -115 to -93°C at night and from -13 to +7°C during the day (Carr 1996). The daily mean temperature at the Martian equator is -58°C (Earth daily mean temperature is +7°C). Because of the thin atmosphere, there is no "greenhouse effect", therefore radiant cooling is extreme at night.

Radiation. Mars is devoid of a strong magnetic field to deflect charged particles from galactic cosmic radiation and solar wind. While the thin atmosphere does provide some protection from this ionizing radiation (Eckart 1996), it is minimal compared to Earth. Because of the thin atmosphere and lack of ozone, ultraviolet radiation is substantially greater on the surface of Mars than on Earth (Salisbury 2000).

Water. Geological and surface topographical features indicate the existence of extensive amounts of free flowing, liquid water on Mars in the past. These include valley networks that are dendritic runoff systems indicative of rain and outflow channels. While there is no liquid water on the surface now, there are estimates that Mars could have had enough water to cover the entire surface of the planet to a depth of 1 km (Carr 1996). Recent reports indicate the possibility of flowing water as recent as 1 million years ago (Malin and Edgett 2000). Potential sources for water on Mars at present include the atmosphere, which could include up to 90 precipitable microns (Jakosky and Farmer 1982), polar

caps (Clifford 1987), deep subsurface water in the form of permafrost (Squyres and Carr 1986) and soil water (Zisk and Mouginis-Mark 1980).

Soil. While we have yet to obtain and analyze samples from Mars, remote sensing data indicate that the Martian soil is 45% SiO_2 and 18% Fe_2O_3 (Smernoff and MacElroy 1989). Elements of potential agricultural importance that could be extracted from the Martian soil or atmosphere include N, Fe, Mg, Ca, K, S, and Si. Not yet reported are the micronutrients B, Mn, Zn, Cu, and Mo (Meyer 1981). The plant macronutrient P is thought to be present (Toulmin et al. 1976). The soils are rich in salts, such as MgSO_4 , NaSO_4 , MgCO_3 , and CaCO_3 (Clark and Van Hart 1981). Terrestrial analogs for soil types and structures that have been suggested include palagonite (a weathering product of volcanic glass) and clays, such as montmorillonite (Singer 1982, Clark et al. 1977).

Gravity. Surface gravity at Mars' equator is 3.73 m s^{-2} , or 0.38 that of Earth (Carr 1996). While there has been little research in the area of plant growth at this g level, indications are that this would be enough to result in normal plants (Brown et al. 1990).

The elements to support plant (and other) life are present on Mars, although at less than optimum levels. Strategies to mitigate these conditions for plant culture include the development of inflatable greenhouses that could function at less than Earth atmospheric pressures (Kennedy 2000) and the use of naturally occurring caves (Boston 2000). The concept of structures to offer some protection from the harsh Martian conditions is feasible and was the subject of a recent workshop and publication (Wheeler and Martin-Brennan 2000). Therefore, our study assumes that in the foreseeable future, plants for life support would be grown in some type of enclosure. While this will provide a modicum of protection, the abiotic stresses experienced by the plants within this structure will be significant, and will require biological and non-biological strategies to survive and thrive.

Nanosensors and Control Devices for Plants and the Environment

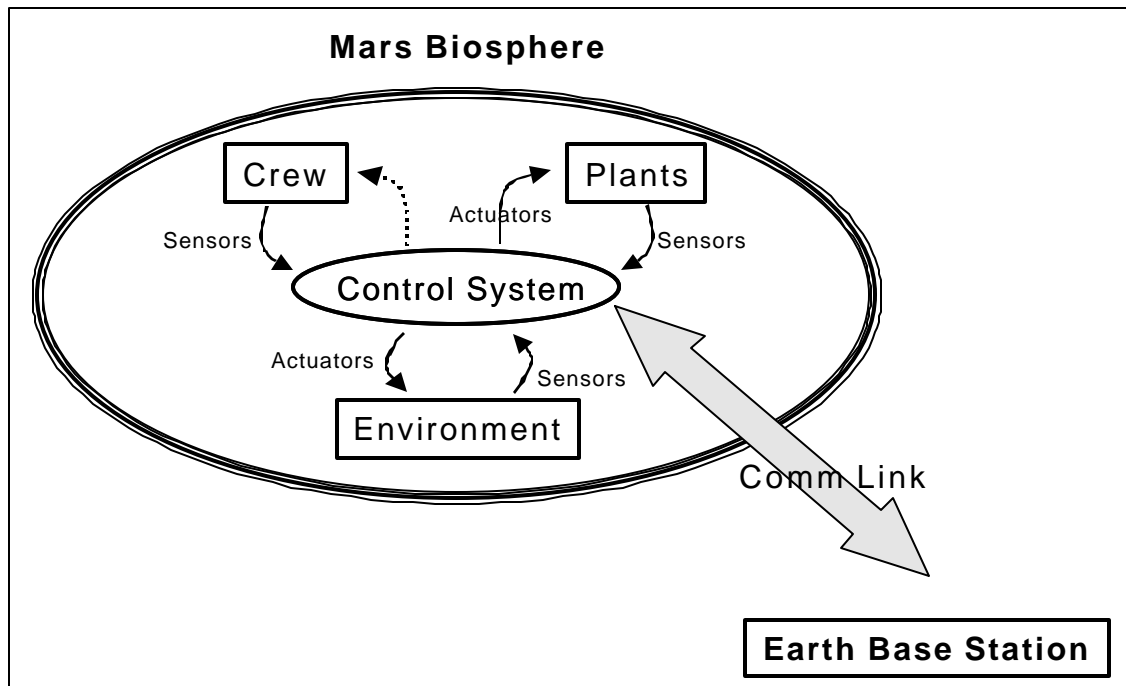
Precise monitoring and control of all aspects of the human/plant/environment biosphere must be a basic system component of the overall architecture for a bioregenerative life support system for human life support on Mars. The integration of nanobiosensors that are linked telemetrically to Earth as part of a distributed intelligent system will be key to success. Because any program of solar system exploration will involve significant periods with humans not present or unavailable to spend time on plant culture the, integration of remotely controlled and smart nanotechnology to monitor and control the function and metabolism of plants will be critical. The distributed sensor system must have versatility and adaptability and be able to monitor both the physiological status of the plants as well as the environment in which they are growing, including the aerial environment and the rhizosphere.

Sensor systems have been developed that enable the automatic monitoring and control of hydroponic nutrient concentrations for plant growth (Morimoto 1992, Balslev et al. 1996). Based on conventional glass electrodes, these sensors operate by potentiometric or polarographic principles to detect oxygen, pH, potassium, phosphate and nitrate. Microfabrication technologies have allowed the integration of these devices into miniature, planarized and multifunctional configurations for the detection of oxygen (Kim 2000), pH (Marzouk 1998), potassium (Cosofret 1995) iron (Milka 1997) phosphate (Engblom 1998) and nitrate (Knoll 1994). While these devices are small and can even accommodate multiple sensor types, they are far from nanoscale devices and little is known regarding their long-term operation, biocompatibility and functionality.

Intelligent control will be a key component of the maintenance of a life support system for Mars. Precise and long-term biological interactions will require human intervention when computer-based control systems begin to drift outside preset tolerance limits. Because of the remote nature of a Mars outpost, human experts will be called on to control these complex systems in real time through fault diagnosis and monitoring. To actively control remote life support systems, machine-learned control systems will need to be developed. This can be accomplished using new smart sensor technology in combination with a thorough understanding of the physiological status of the plant/biosphere.

The overall concept of a remotely controlled biosphere is displayed in Figure 1. This illustrates a biosphere on Mars in which the human inhabitants, the plants, and the environment are linked within a closed system, each depending on the other.

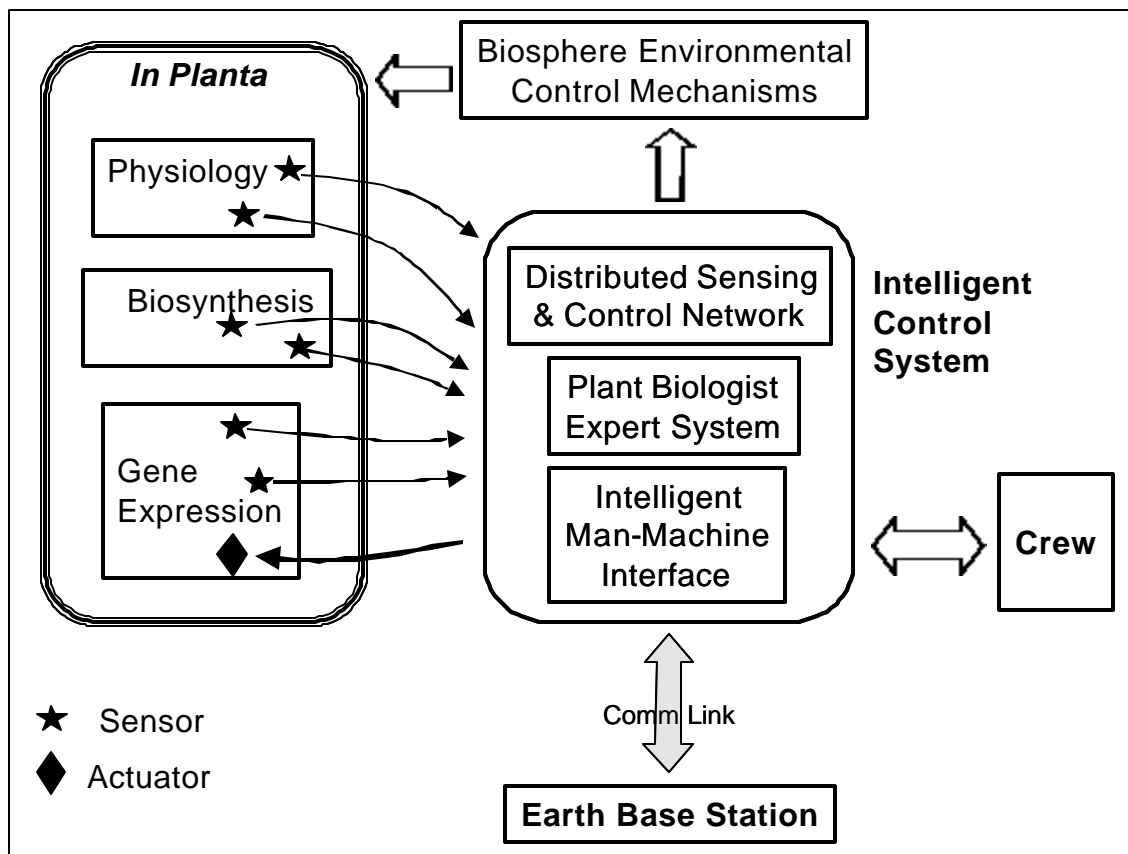
Figure 1. Concept for a Remotely Controlled Mars Biosphere.



The plants, crew, and environment are monitored by a network of sensors which is attached to a central control system. The central control system can be telemetrically monitored from Earth or locally controlled by the resident crew. As new systems software is developed on Earth, new versions may be uploaded to the Martian biosphere. Careful diagnosis of biosphere performance and accurate prediction of potential future system failures will be critical for the success of long-term missions to Mars.

A more detailed depiction of a system of *in planta* control and monitoring is shown in Figure 2. The "stars" indicate a distributed set of implanted sensors for monitoring the physiology and biosynthetic pathways within the living plants. Note that the levels of expression of specific genes will also be monitored. This collective information will be used by the intelligent control system to control both the environment of the biosphere as well as the direct expression of specific genes within one or more species of plants. Actuators for the control of gene expression are denoted by a "diamond" in Figure 2.

Figure 2. Concept of an *in planta* Monitoring and Control System.



Note that the intelligent control system has a plant-biologist expert subsystem as well as an intelligent man-machine interface for the crew. Since the skill set of the crew will be limited, it is unreasonable to expect that any of these individuals will have the needed levels of expertise in plant biology. A computer-based expert system with close communications links to evolving databases on Earth will give the Martian biosphere the best chance for success. Note that the achievement of these goals will require great advances in nanotechnology (including the self-assembly of nanoscale sensors and actuators) and biocompatibility (synergistic interactions of organic and inorganic materials at the molecular level).

Genetic Engineering and Selection of Plants Life Support for Mars

For centuries, hunter-gatherers, farmers and plant biologists have developed new and better-suited varieties of plants. This approach has produced all of the current plants of agronomic importance and continues to be essential in the development of traits that allow plants to grow in environments and produce at levels that have previously been impossible. However, classical breeding, which involves crossbreeding and screening of populations, is labor intensive and slow. Advances in gene sequencing technologies are leading to the complete genome sequence of the model plant *Arabidopsis* and to the complete genome sequencing of many crop plants (Richmond and Somerville 2000), including several under consideration for advanced life support such as rice, wheat, soybean and potato. This information in turn is being used to identify individual genes and whole signal transduction pathways (regulons) that can be manipulated to alter gene expression and plant phenotype. In this way, the ability of plants to withstand abiotic stresses such as drought, salt and cold can be dissected and then systematically manipulated and enhanced (Kasuga 1999).

Biological systems have adapted to survive and thrive in a variety of environments (Table 1). While perhaps not as severe as those which would be encountered on Mars are nonetheless extreme. The strategies developed by these organisms offer solutions to ensuring the survival of organisms on Mars. With our ability to sequence entire genomes, we have the capability to dissect out the genes or gene families responsible for the survival of these organisms. Using biotechnological approaches we will, in the future, be able to confer these traits to plants or other organisms selected for a life support system.

Of particular note is the fact that seeds of many higher plants are resistant to extreme environmental conditions. While in this dormant state they are not growing and producing, nevertheless they are surviving. A more complete understanding of the various genetically determined physiological strategies devised by nature will allow them to be genetically engineered into crop plants for life support. Ultimately, the mechanisms responsible for this ability to endure may be inducible at will in growing plants to ensure survival.

Table 1. Examples of Terrestrial Adaptations to Abiotic Stresses

Stress Condition	Terrestrial Example of Successful Adaptation
Low Light	Shade tolerant species, seeds
Low Atmospheric Pressure	Alpine species, seeds
Low Temperature	Arctic species, Araceae (thermogenic) ¹ , seeds
Drought	Resurrection plant ² , <i>Tortula ruralis</i> ³ , <i>Physcomitrella</i> ³ , seeds
High Radiation	<i>Deinococcus radiodurans</i> ⁴ , seeds

¹ Siedow and Day 2000

² Alamillo et al. 1994

³ Cushman and Bohnert 2000

⁴ White et al. 2000

We are uniquely poised to initiate a long-term program for the selection and creation of plants suited for the (greenhouse-modified) Martian environment. Plants will be genetically engineered for survival, relying on the mechanisms in Table 1 and others. In addition to the traits for survival, traits for enhancement of plant function such as increased harvest index, high nutritional value, production of secondary metabolites for pharmaceuticals, production of building materials and the ability to thrive in Martian soil will be genetically engineered into the plants of choice. This approach will utilize current and future genetic engineering and biotechnological approaches as well as classical breeding and selection techniques. A program such as this will take place on Earth in specially designed chambers to mimic a particular condition or combinations of conditions which eventually will be encountered on Mars. This will lead to plants that can survive and thrive on Mars. At this point the program will shift to a selection/genetic engineering program on Mars itself.

The central concept that distinguishes the breeding of plants on Mars, is the concept of breeding plants from a remote location (utilizing the approaches in Figures 1 and 2). Two criteria are dramatically different from the traditional terrestrial environment, i.e. the partial adaptation to the Martian environment and the need to be able to stop growth or to induce it from a remote location. The strategy would be to carry out recurrent selection in partially controlled environments so that single or multiple cycles of growth could occur and the progress of growth monitored remotely.

There is no single technology that would make such an approach successful. It would require an integrated approach, using gene transfer technology, combined

with "field testing" and cycles of selection to adapt transgenic plants to unusual environments. It would be possible to build in cycles of mutation and transposition to increase variation, again under remote control. It is presumed that addition of genes could improve resistance to extreme conditions, however, it is likely that it will be necessary to induce knockouts for some genes as well. In this way, an inducible mutation system would allow cycles of growth without mutation punctuated by cycles of increased variation.

As an added benefit, such crops may find specialized use in terrestrial environments, or the information gained from such studies could extend the range of some terrestrial crops due to improved resistance to extreme stress. It is also possible that some aspects of remote monitoring could be useful in terrestrial breeding programs. Many industrial breeding programs include field tests in many locations around the world, and remote monitoring of plant physiological state would be generally useful.

In Planta, Heritable Nanodevices

An ultimate, long-term goal of this system is the design programmable plants whose development and metabolism can be controlled from distances of up to millions of miles. One component of this will be comprised of human-designed, biologically-based and heritable devices which can act as receivers, transmitters, sensors and controllers for the purpose of monitoring and directing plant function.

The concept is as follows: The primary receiver will be capable of interpreting telemetrically transmitted information from control centers on Earth or on Mars. The receiver or "receptosome" will be present in all cells, encoded by their DNA, and capable of receiving specific information. The "receptosome" will act as the master switch coupled to several secondary receivers that will be capable of activating pre-selected sets of genes. When the receptosome receives the external stimulus it will direct the biosynthesis, in the appropriate cell types, of specific secondary receiver devices, which will be designed respond to telemetrically or locally generated signals. This type of system (one primary receiver constitutively expressed in each cell, with secondary receivers specifically generated only when needed in individual cells) will minimize energy expenditure and maximize cellular space by not generating secondary receivers until they are needed.

Since human-designed, biologically-based, task-specific primary and secondary receivers have never before been developed, the concept is, indeed, revolutionary. Ultimately, this type of system will depend on advances in the miniaturization to subcellular dimensions of the "receptosome" component parts to nanoscale sizes. Additionally, materials which are biocompatible will need to be incorporated. An understanding of the cellular machinery such that directed biogenesis of intracellular bodies will be required. Finally, and perhaps most difficult to accomplish, will be to determine how to make such a system heritable.

Conclusions and Future Work

With genomics and nanotechnology now established as scientific/engineering disciplines, it is time to amalgamate these fields for the development of organisms that will make up a robust, versatile, and remotely controlled bioregenerative life support system for solar system exploration. The overall architecture and potential time frame for the implementation of this idea is shown in Table 2. Many of the systems and subsystems will progress concomitantly. The five component systems, which have been discussed in this report, are 1) Creation of suitable habitats for Mars; 2) Genetic engineering and selection (on Earth) of plants with traits suitable for Mars; 3) Genetic engineering and selection (on Mars) that will be remotely monitored and controlled; 4) Monitoring and control of Martian biospheres, and perhaps the area requiring the most revolutionary thinking is 5) Man-made, *in planta*, heritable devices for the control and monitoring of plant function.

Any long-term exploration and habitation by humans off of our planet will require biology. The ideas put forth in this report represent the initial conceptual framework that could eventually provide for all the air, water, food, fiber, pharmaceuticals, chemical feedstock, and material needed to sustain the human inhabitants and ensure the overall success of the mission.

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