MELOS LIFE SEARCH PROPOSAL: SEARCH FOR MICROBES ON THE MARS SURFACE WITH SPECIAL INTEREST IN METHANE-OXDIZING BACTERIA. A. Yamagishi[1], Y. Yoshimura[2], H. Honda[3], A. Miyakawa[4], Y. Utsumi[5], T. Itoh[6], T. Naganuma[7], S. Ohno[8], R. Ishimaru[8], S. Sasaki[9], T. Kubota[10], T. Satoh[10], K. Tonokura[11], H. Miyamoto[11], [1]Tokyo University of Pharmacy and Life Science, Hachioji, Tokyo, Japan, [2]Tamagawa University, Tokyo, Japan, [3]Nagaoka University of Technology, Niigata, Japan, [4]Shizuoka University, Shizuoka, Japan, [5]University of Hyogo, Hyogo, Japan, [6]RIKEN BioResource Center, Saitama, Japan, [7]Hiroshima University, Hiroshima, Japan, [8]Chiba Institute of Techonology, Chiba, Japan, [9]NAOJ, Japan, [10]ISAS/JAXA, Kanagawa, Japan, [11]The University of Tokyo, Tokyo, Japan,

Introduction: Among the planets and giant satellites in our solar system, the characteristics of Mars are most similar to those of Earth. This may suggest that it may be possible for life similar to terrestrial life to arise and to survive on Mars.

In the Viking missions conducted by NASA in 1976 the Gas Chromatography / Mass Spectrometer (GCMS) Experiment showed the absence of detectable organic compounds in the upper 10 cm of surface soil[1]. The results of the experiments were interpreted to indicate the presence of oxidants that decomposed the organic compounds, and no organisms were present within the detection limits of the experiments [2].

However, the results of the Viking experiments have been reexamined. Biomolecules such as amino acids could not be detected if living cells were present at levels less than 10^7 cells per gram[3]. Thus some organic compounds may be present on Mars, although compounds near the surface may be destroyed by ionizing radiation[4,5].

The Planetary Fourier Spectrometer onboard the Mars Express spacecraft detected methane at a concentration of approximately 10 ppbv in global abundance[6,7]. Similar abundance was observed by the Fourier Transform Spectrometer at the Canada-France-Hawaii Telescope[8]. Methane was released in large plumes and peak amounts were observed during Martian summer with high-dispersion infrared spectrometers[9].

Here we propose a new life detection project on Mars to search for methane-oxidizing microbes by fluorescence microscopy combined with amino acid analysis and mass spectrometry[10]. We propose to search for “cells” from a depth of about 5 - 10 cm below the surface, which is feasible with current technology. Microscopic observation can be done using low mass equipment with low electric power consumption, and has the potential to detect single “cells”. The subsequent analysis of amino acids will provide the information needed to define the origin of the “cell”.

Survivability of Life in the Mars Environment: Physical and chemical limits for terrestrial life have been major foci in astrobiology[11,12], and are summarized in ref. [10].

UV radiation on the Mars surface can be estimated to be 20 W m^{-2}. If the same shield performance holds for other ionizing radiations[10], ca. 1.2 mGy day^{-1} as determined during the 2001 Mars Odyssey mission, a dose of 0.4 mGy day^{-1} is estimated for the Mars surface. This dose of ionizing radiation is far below the effective dose to kill radio-resistant microbes.

Results of the Viking biological experiments were interpreted to indicate the presence of highly oxidizing compound, which could be generated by photochemical reactions and/or dust storms[13]. Many microorganisms show high tolerance to oxidative conditions, and thus any possible Martian life may not be damaged too seriously[14]. Extended survival of several organisms and aminoacids under simulated martian surface conditions has been reported[15].

What is Needed for Life: “Liquid water” is the simplest and most universal answer. However, many microorganisms can survive in a vacuum for many years. Not only spore forms but also vegetative cells can be stored alive in a vacuum under dessicated conditions.

Another requirement to sustain life is Gibbs free energy[10]. In general, we often refer to it simply as “energy”. Animals depend on food to sustain life. Oxygen is also needed to sustain animal life. These are the two substrates needed to obtain Gibbs free energy for animals. Free energy can be also obtained via other metabolic pathways, such as photosynthesis in plants, and chemosynthesis in some microorganisms. Chemosynthetic microorganisms can obtain free energy from the reaction between reducing compounds and oxidative compouns.

Combining these factors, anywhere in the Martian environment where we can find these three components, water molecules, reducing compounds such as H_2, H_2S and CH_4, and oxidative compounds such as ferric oxide, sulfate and perchlorate, could be an environment where life can be sustained for long periods of time, if other factors such as temperature, pressure, UV and other radiations permit.

Methane Oxidizing Bacteria on Earth: Methane can be used as a primary energy source by a number of

Bacteria and Archaea. Most known methane-oxidizing bacteria are aerobic; however, some evidence, mostly indirect, points to the existence of anaerobic methanotrophs[16]. Recently, a microbial consortium that is capable of using manganese (birnessite) and iron (ferrihydrite) to oxidize methane has been predicted in marine methane-seep sediments in the Eel River Basin in California[17]. Thus, there are several mechanisms of methane oxidation carried out by Bacteria and Archaea on Earth, and possibly on Mars.

How to Find Microbes on Mars: Fluorescent microscopy is a method to detect localized biosignatures *in situ*. Biosignatures are labeled with fluorescent dyes. Stained objects are observed with an epifluorescence microscope with a resolution of 1 μm. This method is highly sensitive. Many fluorescent dyes are commercially available and used routinely to study terrestrial microorganisms[18,19]. Analytical procedures are simple and can be done in a short time. Dye solutions will be sprayed onto samples and digitized images will be obtained using a CCD camera.

The combination of pigments used will be optimized to detect biological characteristics that define life. The "cell" should be surrounded by an impermeable membrane to define "self" and "non-self" and to distinguish inside from outside. The presence of this defining characteristic will be tested detecting the boundaries using a combination of membrane permeable and impermeable pigments.

The second characteristic is metabolism. All life forms depend on free energy, obtained from metabolism. Metabolism, in turn, consists of a complex series of biological reactions called metabolic pathways, which are catalyzed by enzymes. We plan to detect esterase, one of the most commonly found enzymes in cells on Earth.

The third characteristic is division or proliferation of a "cell". Because it is not easy to find appropriate conditions for proliferation, direct observation of the proliferation process is less feasible. Instead, we will target the genetic molecules needed for reproduction of the genetic information.

Upon identification of candidate "cells" by fluorescence microscopy, they will be analyzed by second stage analytical process, possibly in another misson. In the second stage, the "cells" will be hydrolysed.

Living cells on Earth consist of 70% water and 15% protein. Cells contain many types of proteins, from as many as several thousand proteins in prokaryotes, to several tens of thousands in eukaryotes, each having a molecular weight from a few thousand to several hundred thousand. The molecular weight spectra are too complex to be resolved by any type of mass spectral detectors. However, once proteins are hydrolyzed, they produce a mixture of 19 chiral-specific amino acids and glycine, which has no optical isomer. A specific set of 20 amino acids is commonly found in all cells on Earth. Based on research on chemical evolution, which must have occurred before the origin of life, amino acids are known to be abiotically produced in a wide range of possible pre-biological environments. Accordingly, there is a fair chance that Martian "cells" contain polymers of amino acids. However, the number, types and chirality of the amino acids may not be the same as those in living cells on Earth. The number and characteristics of amino acids will be analyzed in the second stage of "cell" analysis.

Conclusion: We propose to search for microbes on Mars, 5 to 10 cm below the surface. The first effort should be to identify locations where methane is emitted from underground. The rover will approach the methane-emitting site, where soil will be collected and analyzed. A combination of fluorescent dyes will be used to detect candidate "cells" using a fluorescence microscope[10]. Possibly in another mission, putative "cells" will be hydrolyzed and analyzed by HPLC and/or mass spectral analysis to define the characteristics of the candidate "cells", which will indicate the origin of the candidate "cells" [10].

References: [1] Biemann, K., et al. (1977) J. Geophys. Res., 82, 4641-4658. [2] Klein, H. P. (1977) J. Geophys. Res. 82, 4677-4680. [3] Glavin, D. P., et al. (2001) Earth Planet. Scie. Lett. 185, 1-5. [4] Dartnell, L. R., et al. (2007) Biogeoscie. 4, 545-558. [5] Kminek, G. and Bada, J. L. (2006) Earth and Planetary Science Letters, 245, 1-5. [6] Formisano, V., et al. (2004) Science, 306, 1758-1761. [7] Geminale, A., et al. (2008) Planet. Space Scie. 56, 1194-1203. [8] Krasnopolsky, V. A., et al. (2004) Icarus, 172, 537-547. [9] Mumma, M. J., et al. (2009) Science, 323, 1041-1045. [10] Yamagishi, A, et al. (2010) Biolo. Scie. Space, 24, 67-82. [11] Rothschild, L.J., and Mancinelli R.L. (2001) Nature, 409, 1092-1101. [12] Marion, G.M. et al. (2003) Astrobiolo. 3, 785-811. [13] Atreya, S.K., et al. (2006) Astrobiol, 6, 439-450. [14] Mancinelli, R. L. (1989) Adv. Space Res. 9, 191-195. [15] Johnson, A. P. et al. (2011) Icarus, 211, 1162-1178. [16] Panganiban, A. T., Jr., et al. (1979) Appl. Enviro, Microbiol. 37, 303-309. [17] Beal, E. J., et al. (2009) Science, 325, 184-187. [18] Herman, B. (1998) Fluorescence microscopy, 2nd ed., Bios Scientific Publishers, Oxford UK. [19] Haugland, R. P. (1996) Handbook of fluorescent probes and research chemicals. 6th ed., Molecular Probes Inc. Eugend OR USA.