

Ancient micronauts: interplanetary transport of microbes by cosmic impacts

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Recent developments in microbiology, geophysics and planetary sciences raise the possibility that the planets in our solar system might not be biologically isolated. Hence, the possibility of lithopanspermia (the interplanetary transport of microbial passengers inside rocks) is presently being re-evaluated, with implications for the origin and evolution of life on Earth and within our solar system. Here, I summarize our current understanding of the physics of impacts, space transport of meteorites, and the potentiality of microorganisms to undergo and survive interplanetary transfer.

Revitalization of an old idea

The possibility that life can move throughout the universe has been a broad topic of philosophical debate for at least the past two millennia (Box 1) [1]. Now the subject can be addressed scientifically upon the recent fusion of astronomy, cosmology, planetary sciences and biology into the emergent discipline of astrobiology [2]. Both theoreticians and experimental scientists are testing the feasibility of natural interplanetary exchange of microbes within rocks as the result of natural impacts – a process dubbed ‘lithopanspermia’ or ‘transpermia’ [3–10].

Considerable experimental effort has been expended in constructing meaningful simulations of various aspects of lithopanspermia and measuring the survival of microorganisms to conditions approximating those prevailing during the process. Because of their intrinsic high resistance to a variety of environmental insults, their ubiquitous global distribution and their amenability to culture and experimental manipulation, spores of *Bacillus* spp. (in particular *B. subtilis*) are the most widely-used model microorganism for lithopanspermia studies [5–7,10]. However, various other model microorganisms have been utilized in such simulations, such as vegetative cells of the soil bacteria *Deinococcus radiodurans* and *Rhodococcus erythropolis*, some halophilic archaea (*Haloerubrum* and *Halobacterium* spp.), the cyanobacterium *Chroococcidiopsis* and the lichens *Xanthoria elegans* and *Rhizocarpon geographicum* [11] (Figure 1). Here, I summarize the current state of our understanding and discuss some of the implications of lithopanspermia theory.

Stages and probabilities

As currently envisioned, the lithopanspermia process can be conveniently divided into several distinct stages: (i) launch of microbe-bearing rocks from the ‘donor’ planet into space; (ii) transit through space from the ‘donor’ planet to the ‘recipient’ planet and (iii) entry and deposition onto the ‘recipient’ planet [3–10] (Figure 2). Because each phase of transfer is fraught with its own unique hazards to microbial life, and because microbes are present in rocks as populations, the probability of surviving the entire process can be broken down into separate factors according to the following relationship (adapted from Refs [8,9]):

$$P_{AB} = P_{BIZ} * P_{EE} * P_{SL} * P_{SS} * P_{SE} * P_{SI} * P_{REL} * P_{SP}$$

where P_{AB} is the probability of a successful transfer from donor planet A to recipient planet B of viable organisms, as the product of the following probabilities:

- P_{BIZ} = probability that the impacting object strikes a biologically inhabited zone;
- P_{EE} = probability of ejection of rocks with endolithic (see Glossary) microbes onto an escape trajectory;
- P_{SL} = probability that an organism survives the launch;
- P_{SS} = probability of survival of space transit;

Glossary

4', 6-diamidino-2-phenolindole (DAPI): a fluorescent stain that binds strongly to DNA forming a blue/cyan complex.

Acridine Orange (AO): a fluorescent dye that binds strongly to nucleic acid, forming a green complex with DNA and a red complex with RNA.

Enceladus: the sixth largest moon of Saturn, containing an icy surface and a probable subsurface liquid water ocean.

Endolithic: literally, “inside rock”.

Escape velocity: the minimum velocity needed to escape a gravitational field; 5.03 and 11.2 km/sec for Mars and Earth, respectively.

Europa: fourth largest moon of Jupiter, containing an icy surface and a probable subsurface ocean of liquid water.

Long Duration Exposure Facility (LDEF): a large cylindrical NASA satellite carrying 57 science and technology missions. LDEF flew in low Earth orbit for 5.7 years, from April 7, 1984 until January 12, 1990.

Magnetic signatures: magnetic minerals, such as magnetite, in a rock can leave behind traces of the rock’s history, such as the magnetic field of the parent planet, or the history of shock and heating during ejection into space.

Petrographic analyses: detailed description of the mineral, textural and chemical features of a rock.

Spallation: a process in which fragments of material (spall) are ejected from a body due to impact or stress.

Terminal velocity: the constant maximum velocity reached by a body falling through an atmosphere under the attraction of gravity, approximately 50 and 300 m/sec for Earth and Mars, respectively.

Box 1. Panspermia from the ancient Greeks to the 20th century

The Greek philosopher Anaxagoras (500–428 BCE) asserted that the seeds of life are present everywhere in the universe – the philosophical starting point of a theory known today broadly as *panspermia* (literally ‘seeds everywhere’). Panspermia theory postulates that life could originate anywhere in the universe where conditions are favorable, and that mechanisms exist for the movement of life from one location to another through space. Thus, the abundant life seen on Earth need not have originated here. Scientific thinking about panspermia began to gain impetus in the 19th century after the chemists Thenard, Vauquelin and Berzelius in the 1830s reported finding organic (carbon-containing) compounds in samples of meteorites fallen from space (see Ref. [1] for a historical review of panspermia). The notion that these carbonaceous materials actually represented living matter inspired the German physician H.E. Richter (1865) to first propose a mechanism for panspermia, in which meteorites glancing the Earth’s atmosphere at a very shallow angle could acquire atmospheric microorganisms before skipping back into space.

Richter’s original notion of meteors as the transfer vehicles for life through space was expanded upon by two of the leading physicists of the age, Hermann von Helmholtz and William Thomson (Lord Kelvin). In 1871, each proposed a hypothesis that outlined many details of what has since become known by various names such as *lithopanspermia* (‘rock panspermia’), *ballistic panspermia* or *transpermia*, in addition to a mechanism by which transfer might work: cosmic impacts. Thomson proposed that bodies impacting a living planet like Earth could blast life-bearing rocks into space, and that similar meteorites blasted off other living worlds might have inoculated the early Earth with life. In addition to meteorites, von Helmholtz included comets as putative vehicles and proposed a key prediction of lithopanspermia – that organisms arising from the donor and recipient planets would share a common ancestry. An alternative mechanism for panspermia was later proposed by Swedish chemist and Nobel laureate Svante Arrhenius (1903), by which spores could be transported through space by the radiation pressure emitted from stars (‘radiopanspermia’). We now know that the intense ultraviolet light from solar radiation in space is rapidly lethal to unshielded microorganisms, but Arrhenius nonetheless did much to popularize the notion of panspermia in the early 20th century through articles, popular books and public lectures on the subject. In the mid-20th century, Hoyle and Wickramasinghe proposed a not-widely-accepted cyclical version of panspermia, in which they postulated that interstellar dust grains were actually viable microorganisms that were amplified in the ‘warm, watery interiors’ of comets, then delivered to planets by cometary impacts and outgassing. According to this theory, after further amplification on the planets, the resulting viable biological material was then returned to interstellar space to start the cycle anew [75].

Almost from the moment of its proposal, the lithopanspermia hypothesis drew intense criticism – at the time it was thought that living organisms could not possibly survive ejection by impact, transit through space and entry onto another planet. These views held sway until the discovery on Earth and characterization of meteorites from Mars in the late 20th century.

P_{SE} = probability of surviving entry through the recipient planet’s atmosphere;

P_{SI} = probability of surviving impact onto the recipient planet’s surface;

P_{REL} = probability of release from the interior of the rock;

P_{SP} = probability of survival and proliferation in the environment of the recipient planet.

In order for interplanetary transfer to be ecologically relevant, some fraction of an initial microbial population (or microbial community? or micro-ecosystem?) would have

to surmount each of the barriers just described. Of course, some of the terms in the aforementioned equation would be difficult, if not impossible, to determine accurately; indeed, different groups analyzed essentially the same equation and available dataset and came to opposite conclusions – that interplanetary transport of microbes was either highly probable [9] or highly improbable [8].

Candidate microbes for impact-mediated launch (P_{BIZ})

Historically, one of the main arguments against lithopanspermia was that the energies required to eject rocks from the surface of a planet into space would be so high as to partially melt or even vaporize the rock, thus rendering it sterile [8,9]. However, since the late 1970s it has been recognized that several meteorites found on Earth are actually bits of crust derived from the Moon and Mars [12], and examination of their heat-labile carbonates and magnetic signatures indicated that many of the Martian meteorites had been boosted into space suffering only rather light shock pressures. Indeed, some Martian meteorites were never heated above $\sim 100^\circ\text{C}$ [13,14].

How could these natural samples of the Martian crust have been transferred to Earth? Recent advances in the physics of impacts have provided insights into how rocks can be launched into space with relatively little damage. A considerable amount of theoretical and experimental support has accumulated, favoring a spallation mechanism for impact ejection [15–18]. In this mechanism, a transient spallation zone forms around an impact site, where the reflected shock wave of the impact is directly translated into acceleration of surface rocks to escape velocity. Because the spallation zone penetrates at most only a few meters into the surface, and because most of the Martian meteorites are igneous (mainly basalt), it becomes relevant to understand the microbial ecology of near-surface igneous rocks on Earth (basalts and granites) which might harbor potential microbial candidates for interplanetary transfer.

Although relatively little is known regarding the ecology of endolithic microbes, some of the few studies performed are instructive. In one study, eleven deep-subsurface rock samples were collected from beneath Rainier Mesa, Nevada Test Site (USA), and the numbers of cultivable microorganisms were found to range widely, from <10 to $>10^5$ per gram [19]. Direct counts of these same rock samples using fluorescent staining with acridine orange (AO) or 4', 6-diamidino-2-phenolindole (DAPI) revealed $\sim 4 \times 10^5$ to $\sim 4 \times 10^7$ total microbes per gram of rock [19]. From these data, the total number of microbes in rock exceeds the viable count by up to 2–3 orders of magnitude. In another study, culturable *Bacillus* spores were found at very low numbers (~ 10 per gram) when sampling near-subsurface basalt [20]; however, higher numbers of cultivable spores were obtained from the interior of near-subsurface granite (5×10^2 spores per gram out of $\sim 10^4$ total cultivable bacteria per gram) [21]. Interestingly, these endolithic isolates were very closely related to a limited number of *Bacillus* species previously found to inhabit globally distributed endolithic sites (biodeteriorated murals, stone tombs, underground caverns and rock concretions) and extreme environments

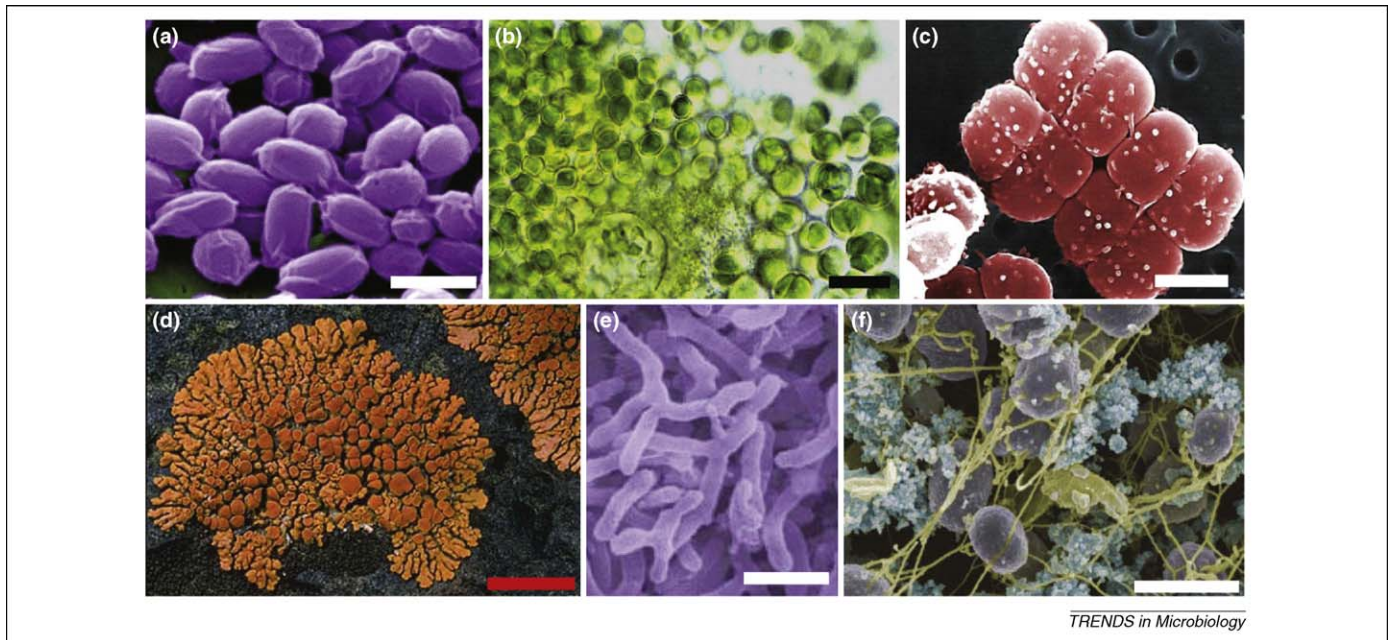


Figure 1. Some of the model microorganisms used in lithopanspermia simulations. (a) *Bacillus* sp. spores (false-color SEM image. Photo credit: Janice Hanley Carr). (b) The cyanobacterium *Chroococcidiopsis* (light micrograph by E.I. Friedmann and R. Ocampo-Friedmann). (c) *Deinococcus radiodurans* (false-color SEM image, by Peggy A. O’Cain and Margaret C. Henk, Louisiana State; modified by Peter Reid, The University of Edinburgh). (d) The lichen *Xanthoria elegans* (macroscopic colony on rock; image kindly provided by Stephen Sharnoff). (e) The Gram-positive nocardioform actinomycete *Rhodococcus erythropolis* (scanning electron micrograph by Ernesto Plagiario). (f) The halophilic archaeon *Halorubrum* (false-color SEM image kindly provided by Shil DasSarma). Scale bars are: 1 μm (white), 10 μm (black), 1 cm (red).

(Antarctic soils, deep sea floor sediments and spacecraft assembly facilities) [20,21]. Thus, it seems that the occurrence of *Bacillus* species in endolithic or extreme environments is not accidental, but these environments seem to create unique niches to which a limited number of *Bacillus* species are specifically adapted [20,21].

Surviving launch (P_{SL})

The most important physical forces to which hypothetical endolithic microbial passengers would be subjected during launch by impact are acceleration, compression shock and heating. What are the magnitudes of these forces? Petrographic analyses of the collection of Martian meteorites

indicate that during ejection from Mars these rocks experienced shock in the range from ~ 5 GPa to ~ 55 GPa [22–27] and heating in the range from ~ 40 °C to ~ 350 °C [13,14]. Mathematical modeling of impact events suggests that, to reach Mars escape velocity (5.03 km/sec), the ejected materials would be subjected simultaneously to acceleration on the order of $\sim 3.8 \times 10^6$ m/sec² (~ 390 000 xg) and ‘jerk’ (i.e. the rate of change of acceleration) of $\sim 6 \times 10^9$ m/sec³ [28]. These forces have been replicated singly or in combination using a variety of simulation experiments in which model microbes have been subjected to ultracentrifugation, hypervelocity ballistic experiments and static explosive compressional shock and heating.

Ultracentrifugation and low-speed ballistics experiments demonstrated that spores of *B. subtilis* and vegetative cells of *D. radiodurans* were relatively unaffected by the acceleration and jerk components characteristic of launch from Mars, applied without shock or heating [28]. Using explosive compression experiments, survival of three microbial species to various shock pressures and heating (spanning the known shock pressure values of the Martian meteorites) was measured in the absence of acceleration or jerk [23,28,29]. These results showed that shock and heating were lethal to all three species, and dose–response inactivation curves were constructed relating survival to shock pressure. Spores of *B. subtilis*, and the photobiont and mycobiont partners of the lichen *X. elegans* survived on the order of $\sim 10^{-4}$ when confronted with shock pressures up to 40–50 GPa, whereas no surviving cells of the cyanobacterium *Chroococcidiopsis* were recovered from shock pressures higher than 10 GPa [27,29]. Hypervelocity impacts in which microbe-laden projectiles were fired into agar or ice targets also resulted in dose–response curves relating survival of bacterial cells or spores to the

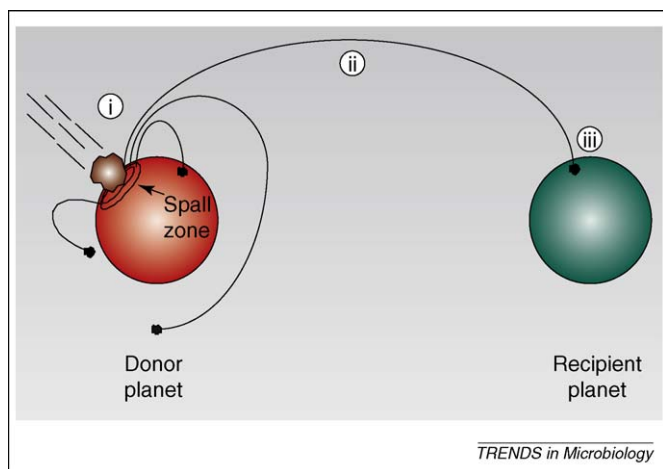


Figure 2. Stages in lithopanspermia. (i) An asteroid or comet strikes the donor planet, ejecting near-surface rocks from the spallation zone. (ii) A fraction of the rocks are accelerated to escape velocity and travel through space on a trajectory ultimately intersecting the recipient planet. (iii) The rocks are captured by the gravity of the recipient planet, enter the atmosphere and fall to impact the surface.

shock pressures calculated to occur in the experimental impacts [30]. In this study, *B. subtilis* cells and spores were reported to survive hypervelocity (4.9–5.4 km/sec) impacts generating 67–78 GPa at a frequency of 10^{-5} to 10^{-4} , and *Rhodococcus erythropolis* cells survived at 10^{-7} to 10^{-5} [30].

Because considerable experimental and theoretical evidence supports a spallation mechanism for launch, an experimental simulation of launch by spallation was devised using hypervelocity ballistics [31]. As a proxy for near-subsurface endolithic bacteria, *B. subtilis* spores were applied to the surface of a granite target, which was struck perpendicularly from above by an aluminum projectile fired at 5.4 km/sec. Granite fragments spalled upward from the target were recovered and assayed for shock damage by transmission electron microscopy and for spore survival by viability assays. Shock pressure at the impact site was calculated to be 57.1 GPa; however, recovered spall fragments were only very lightly shocked at pressures of only ~5–7 GPa. Spore survival was calculated to be in the order of 10^{-5} , in agreement with results of previous static compressional shock experiments [31]. From the data, it was calculated that rock ejected into space by an impact could contain between 50 and 5×10^6 spore survivors per kg, and that even small impacts can eject on the order of 10^8 – 10^9 kg of rock into space [32,33]. Therefore, it is likely that a hypervelocity impact could launch considerable numbers of viable microbes into space, lending further evidence in favor of lithopanspermia theory.

How does shock damage or inactivate cells during impact-mediated launch? As might be expected, microscopic examination of post-shock samples of unstained and live/dead-stained lichen and cyanobacterium samples revealed considerable cell rupture [29]. DNA has also been identified as a target of damage in compression shock experiments because: (i) exposure of *B. subtilis* spores to shock led to production of mutations; and (ii) spores of strains lacking DNA-protective α/β -type small, acid-soluble spore proteins (SASP), or double-strand break repair by non-homologous end joining (NHEJ), were significantly more sensitive to shock than were wild-type spores [34]. The unavoidable heating associated with these shock experiments also had a role, as samples shocked at -78°C suffered about half as many mutations as samples shocked at $+20^\circ\text{C}$ [34].

Surviving space transit (P_{SS})

Once boosted into space, endolithic microbes would be subjected to an entirely different set of environmental stresses, namely extreme vacuum, desiccation, solar and cosmic radiation, microgravity and both extreme hot and cold temperatures. Of these factors, solar UV is the most immediately lethal, but is relatively easily shielded [35]. Spaceflight experiments have shown that with minimal UV shielding several types of bacteria, archaea and viruses can survive at least short-term (weeks to months) exposure to space in low Earth orbit; most notable among these are *B. subtilis* spores, which survived nearly 6 years exposed to full space conditions in Earth orbit on the Long Duration Exposure Facility (LDEF) [10,36]. An important cellular target of solar UV is DNA, and microbes possess several

mechanisms for preventing and repairing UV damage in DNA (reviewed extensively in Refs [10,35]). The types of cellular damage induced by space exposure are largely unknown, with the exception of DNA damage. DNA was identified early as a target of damage by the increased frequencies of reversion to prototrophy in space-exposed *B. subtilis* spores [37]. Exposure to ultrahigh vacuum (UHV) on the order of 10^{-5} to 10^{-7} Pa leads to changes in spore DNA structure as evidenced by the altered UV photochemistry of DNA [38–40] and the altered spectrum of mutations in the *gyrA* and *rpoB* genes [41–43] of UHV-exposed *B. subtilis* spores. In addition, UHV has been documented to cause single-strand breaks (SSB) and double-strand breaks (DSB) in DNA of *B. subtilis* spores and *D. radiodurans* cells [44].

In interplanetary space, outside Earth's protective magnetic field, microbe-containing rocks would be exposed to bombardment by high-energy ionizing radiation from galactic sources and from the sun, consisting of photons (X-rays, γ -rays), protons, electrons and heavy, high-energy atomic nuclei (HZE particles) [45]. Ionizing radiation induces a variety of damage in DNA, including SSB and DSB, as a result of both direct collision and indirect formation of reactive species such as oxygen radicals [46]. DSB in DNA of *B. subtilis* spores are repaired during germination by the NHEJ repair system encoded by the genes *ykoU* and *ykoV* [46,47]. It has been shown recently that DNA repair during germination by NHEJ is important for *B. subtilis* spore resistance to UHV, ionizing photons and HZE particle bombardment [46,47]. In addition, α/β -type SASPs, which bind to spore DNA and protect it from a variety of insults [48], were found to be important determinants of spore survival to simulated space conditions [46].

In the space environment, microorganisms are completely desiccated by the extreme vacuum and unable to maintain metabolic and cellular repair activities, leading to inexorable degradation of their biological molecules and stochastic inactivation [7,9,10]. Under these conditions, transit time in space becomes an important factor. The rather small collection of Martian meteorites spent from ~0.6 to 15 million years in space traveling from Mars to Earth [49,50]. Inactivation models using thermal and radiation inactivation kinetics suggest that these extremely long transit times limit the probability of viable microbial transfer [45,51]. However, modeling of the trajectories of materials ejected by impacts reveals the possibility that a small percentage of meteorites can be launched on fast-transit trajectories (a few months to a few years) between Earth and Mars [52].

Several currently ongoing astrobiology experiments are located on the outside of the International Space Station on two exposure facilities, EXPOSE-E and EXPOSE-R. These experiments are testing a variety of microorganisms, and plant seeds, for their responses to long-term exposure to the environment of space (for more details, see http://www.esa.int/esaHS/SEMAVT9WYNF_research_0.html). In addition to viability, upon return these experiments will be assaying cellular responses to space exposure such as DNA damage, mutagenesis and transcriptional responses during spore germination.

Surviving atmospheric entry and landing (P_{SE} , P_{SI} , P_{REL})

If a rock approaches close enough to a recipient planet, it will be attracted by that planet's gravity field and fall through the atmosphere onto the surface. The physics of high-speed meteorite passage through the atmosphere is quite well understood [53,54], but the survival of microbes during this last phase of transfer has received relatively little experimental attention. Meteorites enter the upper atmosphere at considerable speeds of 10–20 km/sec. Frictional heating melts the surface of the meteorite and aerodynamic forces ablate the liquid rock, carrying away the heat with it. The short transit time of meteorites through the atmosphere (a few tens of seconds) precludes penetration of heat more than a few millimeters into the interior of the rock; therefore, apart from a thin (<1 mm) fusion crust at the surface, the interiors of meteorites are not strongly heated [14,53]. Aerodynamic drag forces often disaggregate the rock in the lower atmosphere, and the resulting fragments strike Earth's surface in the characteristic pattern of a strewn field [54]. On planets with atmospheres, impact with the surface at the end of the flight occurs at terminal velocity, which is roughly 50 m/sec for Earth and 300 m/sec for Mars; therefore, the forces generated during final impact would be quite modest compared to those prevailing at launch (see earlier). Upon impact, the rock fragments are further shattered and mixed with the surface material of the destination planet. It is therefore possible not only for endolithic microbes to survive re-entry embedded in a meteorite but also to actually be effectively released into the recipient planet's crust or water, thereby encountering an environment potentially conducive to growth [10].

Experimentally, it is difficult to simulate hypervelocity re-entry from space. Using sounding rockets, it was demonstrated that substantial numbers (up to 13%) of *B. subtilis* spores infused into the surface of artificial granite meteorites were able to survive ballistic space-flight (including two high-speed atmospheric passages of 1–2 km/sec during ascent and descent), suggesting that microbes buried deeper within rocks would probably survive the entry phase in high numbers [53]. A higher atmospheric entry velocity (7.7 km/sec) was obtained in the STONE-5 and STONE-6 experiments, in which spores of *B. subtilis* and the fungus *Ulocladium atrum*, and vegetative cells of lichens and the cyanobacterium *Chroococcidiopsis*, were loaded into various rock samples mounted on the heat shield of a FOTON-M2 unmanned recoverable orbital capsule. No viable microorganisms were recovered from any of the samples, indicating that lethal levels of heating had penetrated at least 1 cm into the rocks (or alternatively that hot gases had leaked underneath the samples) [55–57]. Biological investigation of the *Chroococcidiopsis*-containing sample showed that the heat of entry had ablated and heated the original rock to a temperature well above the upper temperature limit for life to below the depth at which light levels are insufficient for photosynthetic organisms (~5 mm), thus killing all of its photosynthetic inhabitants. The results suggested that atmospheric transit could act as a strong biogeographical dispersal filter to

the interplanetary transfer of photosynthetic organisms, which must be located near the surface of rocks [3,55].

Survival and proliferation on the recipient planet (P_{SP})

For microbial cross-contamination of planets to be ecologically relevant, upon arrival the interplanetary travelers must be able to survive, disperse and proliferate in their new environment [3,5,8,10]. The present-day environments of neighboring planets such as Earth and Mars are radically different [5,58], and a key challenge of lithopanspermia theory is the experimental testing of the limits of life both in extreme terrestrial environments and in accurate simulations of the Martian surface and subsurface.

The Martian environment presents several obstacles which could be potentially lethal or at least inhibitory to Earth microbes, such as: a highly biocidal short-wave solar UV flux, a low pressure atmosphere dominated by CO₂, scarcity of liquid water, low or absent organic nutrients, extreme cold and extreme temperature changes, and potentially highly-oxidizing soils, to name but a few [5,58]. Recent experiments have been directed towards testing the ability of several bacterial species to survive and grow under simulated Mars environmental conditions. The results indicate that the UV flux at the Martian surface is rapidly lethal to vegetative bacteria and spores [5,58–63]. A lack of organic nutrients in Mars soil would prevent the growth of heterotrophic organisms, however autotrophs such as methanogens have been shown to be metabolically active in simulated Mars soil hydrated with buffer, and produced methane and biomass from CO₂ [64]. However, these experiments were not performed under realistic Martian environmental conditions. Another inhibitory component of the Martian environment is low pressure: the atmospheric pressure on the surface of Mars ranges from ~10² Pa at the top of Olympus Mons to ~10³ Pa at the bottom of Hellas basin, whereas Earth's atmosphere averages ~10⁵ Pa at sea level [58]. Exposure to pressures below ~5 × 10³ Pa reversibly inhibited the growth of a variety of microorganisms [65,66], and the combination of exposure to cold temperatures and a CO₂-dominated atmosphere raised the low-pressure threshold considerably [65,66]. Thus, harsh Mars surface conditions coupled with the scarcity of liquid water and nutrients poses a formidable barrier to the survival and proliferation of Earth microbes at the surface of present-day Mars.

The combination of temperature and pressure on Mars poses an interesting astrobiological puzzle. Temperatures at the Martian surface are usually below 0 °C and pressures are close to the triple-point vapor pressure of water (6.1 mbar), thus there is only a very narrow range of temperatures at which pure water might be found in the liquid state [67]. However, the finding of salts and evaporitic minerals on the Martian surface [68] suggests the past existence of highly saline water (brines). Salts such as those found on Mars have been shown to depress the freezing point of water such that liquid brines could be stable at or near the Martian surface [67]. It stands to reason that to proliferate under these conditions, a microorganism would have to be both a halophile (salt-loving) and a psychrophile (cold-loving). Such microorganisms do

exist on Earth, such as the archaeum *Halorubrum lacusprofundi*, isolated from an Antarctic hypersaline evaporation pond that remains liquid at temperatures below 0 °C, and which has been shown to grow at temperatures down to -1 °C [69,70].

An area of active present and future research is aimed towards understanding the factors that limit the survival and growth of terrestrial microbes on other planets, especially Mars. Current questions being addressed are: what are the cellular targets of growth inhibition when cells are exposed to simulations of Mars pressure, temperature and atmospheric composition? Do microbes exist in extreme environments on Earth that can cope with these conditions? Can Earth microbes adapt and evolve to grow in the Mars environment, and what alterations are necessary? The results of these experiments have implications not only for Planetary Protection (Box 2) but will also guide the design of life-detection missions to destinations such as Mars, Europa or Enceladus, and will lead to a deeper understanding of the conditions under which life can occur in the universe.

Box 2. Humans as agents of interplanetary transfer

Although natural transfer of microbes between planets could have been occurring for billions of years, only within the past half-century have humans begun to leave Earth and to expand their presence onto other planets and moons within the solar system. Since 1962, nearly 40 robotic missions have been launched with Mars as the destination. Many of these probes either missed Mars, crashed onto the Martian surface, successfully landed or landed and then drove over the Martian terrain. Because microorganisms are ubiquitous in Earth's environment, human and robotic missions leaving Earth to explore space carry along a large host of terrestrial microorganisms as accidental contaminants of spacecraft surfaces, and also as integral and beneficial components of the astronauts themselves. The stresses placed upon microorganisms during transfer by human and robotic spaceflight are much gentler than those imposed by natural impacts; hence the probability of microbes surviving human-mediated interplanetary transfer is much greater. Because at least three exploration targets (Mars, Europa and Enceladus) might harbor evidence of past or present life, one of the most important challenges facing mission planners and explorers alike is the protection of these pristine environments from 'forward contamination' by terrestrial microorganisms, and 'back contamination' of Earth environments by potential extra-terrestrial microbes (for a review, see Ref. [76]).

Box 3. Solar system, planetary and biological evolution

The origin and evolution of life on Earth is inextricably linked to the origin and evolution of the solar system and planet Earth itself. Currently it is thought that the solar system arose by gravitational condensation from an interstellar gas cloud called the solar nebula (reviewed in Ref. [77]). The contracting nebula began to rotate and assumed the shape of a central protostar surrounded by a protoplanetary disk, from which the sun and planets formed by further gravitational collapse. During the final phase of planetary formation, up until ~3.8 billion years ago, the increasing gravity fields of the new growing planets, coupled with migration of planets into their present orbits, swept up local debris and perturbed the orbits of smaller asteroids and comets, thus showering themselves and each other with a cascade of impacting objects during a period known as the Heavy Bombardment. It is thought that during the Heavy Bombardment, a large fraction of Earth's water and crucial prebiotic organic compounds might have been delivered from the outer solar system by such impacting objects.

It has been noted that the earliest traces of life on Earth date all the way back to ~3.5 billion years ago, and these most ancient microfossils seem to be morphologically indistinguishable from present-day microbes [78,79]. The apparently short span of time between cooling of the Earth to the point that liquid water could exist and the appearance of such modern-looking microbes has been taken by some scientists as evidence supporting the transfer of these microbes to Earth from elsewhere and their deposition into the primordial soup.

Where could these organisms have come from, if not Earth? Current evidence suggests that in the early days of the solar system, the environments of Venus, Earth and Mars were much more similar than they are today. Each planet possessed a relatively warm environment with abundant liquid water and an atmosphere dominated by CO₂, conducive to the origin of life. Because this period coincided with the latter part of the Heavy Bombardment, it has been speculated that early life-bearing rocks were actively being exchanged throughout the solar system, particularly among the inner planets. However, over the next 4 billion years or so, Venus, Earth and Mars embarked on divergent evolutionary pathways leading to their present radically distinct environments. The accumulated evidence leads us to the following scenario, although others are certainly possible. Early in the history of our solar system, somewhere around 4 billion years ago, the early terrestrial planets (Venus, Earth and Mars) were accreting as a result of impact bombardment. During this period, water and

prebiotic organic compounds were being regularly delivered from the outer solar system by comets and asteroids, but the energy released by their impacts probably kept the planetary surfaces too hot for liquid water to form. Bombardment continued, but the impact rate dropped to the point that eventually each planetary surface cooled to a temperature at which liquid water could exist [80]. The resulting torrential rains filled Earth's present-day oceans, and presumably those of Mars and Venus. On one or more of the terrestrial planets (which one, no one can say), life probably arose in contact with liquid water, heat and nutrients, perhaps in a subterranean or submarine hydrothermal system. Exactly how this could have happened is currently the subject of intense debate (see Ref. [81] and references therein). Some scientists think that, during the period when life was gaining a toehold, occasionally the terrestrial planets were struck by leftover impacting objects that were large and energetic enough to actually boil away the oceans again into the atmosphere – these have been called 'sterilizing' impacts. After each sterilizing impact, the planet cooled again, and water condensed and rained back into the oceans. Eventually the supply of sterilizing impacting objects was exhausted.

The molecular phylogenetic tree suggests that the earliest life forms might have been anaerobic thermophilic microorganisms (although this is also the subject of intense debate, see Ref. [82]), implying that life might have originated in a hot ocean, or in a subterranean or submarine hydrothermal system similar to those found today in 'black smokers' on the ocean floor or in deep hot spring systems such as those in Yellowstone National Park. Alternatively, some scientists speculate that these ancient microbial lineages merely represent the survivors of the last sterilizing impact, which were situated in protected refuges, perhaps near hydrothermal vents on the ocean floor or in deep subsurface hydrothermal systems.

An intriguing twist on the theory of lithopanspermia has been suggested as a mechanism for refuge of microorganisms to sterilizing impacts [83]. In this scenario, a sterilizing impacting object could have blasted large quantities of microbe-bearing surface rocks into space, and that many of these rocks lacked the velocity to completely escape Earth's gravity. These rocks would be placed into decaying orbits, eventually showering back onto Earth over decades, hundreds or thousands of years. As the Earth cooled, water condensed and the oceans reformed in the years following a sterilizing impact, Earth could once again be inoculated by microbe-bearing meteorites, but of Earthly origin.

Concluding remarks

During the early years of solar system evolution, the ancient environments of the terrestrial planets (Venus, Earth and Mars) could have been much more similar than they are today (Box 3). Indeed, evidence has been steadily accumulating that ancient Mars had a thicker atmosphere and a warmer and wetter past [71–73]. The early period in the evolution of Earth and Mars and the estimated time of the origin of life on Earth coincided roughly with the final stages of planetary accretion, the period of heavy bombardment, when there was active exchange of ejected surface rocks between the inner planets. These observations have sparked the speculation that, if life originated on Earth or Mars, it could have spread to the other planet by natural impact processes. This scenario predicts that life on Mars, if it exists today, could share its ancestry with present-day Earth life, hence might be demonstrably related genetically and biochemically. Indeed, currently planned life detection mission experiments rest on the basic assumption that present or past Mars life will leave traces recognizable by terrestrial biochemists and microbiologists [74]. However, since the time of their formation, Earth and Mars have embarked on divergent evolutionary pathways to the point that their present environments are dramatically different. It stands to reason that Earth and (putative) Mars life also diverged over time to adapt to the changing environmental conditions on their respective planets.

After a journey of nearly 12 million years in space, one of the most famous Martian meteorites, Nakhla, fell on June 28, 1911 in northern Egypt [49,50]. Therefore, meteorites from Mars continue to fall on Earth to the present day, albeit at a rate much reduced from the period of heavy bombardment. Current results suggest that the terrestrial microbes tested to date (mostly mesophilic laboratory strains) encounter severe difficulties surviving and proliferating in the present-day Mars surface environment [65,66]; the logical extension of this reasoning is that Mars microorganisms might also have a difficult time prospering in today's Earth environment. It must be kept in mind, however, that we have not yet completely defined the extreme limits of Earth life. In addition, we have only begun to lightly scratch at the surface of Mars in search of habitable conditions and evidence of past or present life (Box 2). Such life, if it ever existed, would almost certainly be microbial in nature. These are times for microbiologists exploring our solar system.

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