# **References (some quotations included to assist verification)**

[Uses Harvard reference style, but in this draft, instead of a, b, c etc., I use unique ids like [(NASA, 2020tesgs](#kix.76qmy7dxcqdq)[)](#kix.6aisxl7zz0qc) - the idea is to search / replace these ids with a, b, c etc once the list is complete, after peer review]

## A

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*Approximately every 28 days, fully differentiated cuboidal basal keratinocytes with large nuclei, abundant organelles, and a phospholipid membrane migrate apically from the basal layer through the spinous and granular layers [4]. During this turnover process, an accumulation of keratin and lipids ensues which then undergoes terminal differentiation to form the stratum corneum  
…  
Skin is an active immunological organ, and dysfunctional innate defenses have serious clinical implications. Products of the stratum corneum, including free fatty acids, polar lipids, and glycosphingolipids accumulate in the intercellular spaces and horny layer, exhibiting antimicrobial properties, and functioning as a first line of defense. Antimicrobial peptides (AMPs) exhibit potent and targeted resistance against a wide spectrum of common pathogens. When this barrier is breached, second lines of protection are provided by inflammatory cascades in the subepithelial tissue. Approximately sixteen AMPs have been shown to be expressed in the skin (Table 1)*

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American Chemical Society, 2015, [“Cyborg bacteria outperform plants when turning sunlight into useful compounds”](https://phys.org/news/2017-08-cyborg-bacteria-outperform-sunlight-compounds.html), Phys.org

*"A future direction, if this phenomenon exists in nature, would be to bioprospect for these organisms and put them to use,"*

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*On Earth, photosynthesis and respiration by living things cause tiny fluctuations in our planet’s otherwise steady oxygen concentration. We shouldn’t expect this on Mars, though. “That’s far out,” Telling says: Mars appears too inhospitable for a critical mass of life capable of sustaining either process. “It’s almost certainly going to be a nonbiological chemical reaction.”*

*Trainer herself does not rule out a biological explanation, but nevertheless underscores its unlikeliness. “People in the community like to say that it will be the explanation of last resort, because that would be so monumental,” she says. There are abiotic mechanisms aplenty, both known and unknown, to rule out first before leaping to any more sensational claims.*

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*A single U.S. facility ticking all of these boxes could cost around $500 million, Dreier says. And it is not yet clear if others will be built in Europe*

*...*

*MSR’s masters are foregoing parachutes because the devices cannot be guaranteed to work, Vijendran says—something immortalized in 2004 by the solar-wind-particle-gathering Genesis mission, whose sample capsule broke open after an unintentional hard landing. In this case, it is simpler to build a rigid capsule that can withstand such a landing. “It just comes in, and, wham, it hits the ground,” Vago says. “That’s going to be an interesting one.”*

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## B

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*"Two strategies have been suggested for seeking signs of life on Mars: The aggressive robotic pursuit of biosignatures with increasingly sophisticated instrumentation vs. the return of samples to Earth (MSR). While the former strategy, typified by the Mars Science Laboratory (MSL), has proven to be painfully expensive, the latter is likely to cripple all other activities within the Mars program, adversely impact the entire Planetary Science program, and discourage young researchers from entering the field."*

*"In this White Paper we argue that it is not yet time to start down the MSR path. We have by no means exhausted our quiver of tools, and we do not yet know enough to intelligently select samples for possible return. In the best possible scenario, advanced instrumentation would identify biomarkers and define for us the nature of potential sample to be returned. In the worst scenario, we would mortgage the exploration program to return an arbitrary sample that proves to be as ambiguous with respect to the search for life as ALH84001."*

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*Some clusters of dozens of diatoms appear pristine, suggesting that they had been living in the salar pool immediately before being trapped as the gypsum crystal grew.*

*Could microfossils and/or viable microorganisms be trapped in gypsum on Mars as they are in gypsum on Earth? It is likely that abundant sulfate sand grains on Mars contain fluid inclusions similar to those in the acid-precipitated bottom-growth and reworked gypsum we discuss here.*

*We suggest that gypsum on Mars would have entrapped, as solid inclusions and within fluid inclusions, any microorganisms and/or organic compounds that were present in its parent waters. Therefore, fluid inclusions and solid inclusions hosted by salt minerals may be the best place to continue the search for life on Mars.*

*Some of these entrapped microorganisms remain viable for at* 2016. [The cosmic zoo: the (near) inevitability of the evolution of complex, macroscopic life](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5041001/). Life, 6(3), p.25.

*Photosynthesis is primarily useful for providing energy for the reduction of environmental carbon ...*

*There are six known pathways for fixing carbon dioxide, of which the Calvin Cycle used in oxygenic phototrophs is the least efficient in terms of the energy and the reducing equivalents (electrons) required per mole of fixed CO₂ ...*

*However, the great advantage provided by oxygenesis was its capacity to liberate life from the need to find rare electron donors such as sulphide, hydrogen or Fe(II) to support the reduction of carbon dioxide, giving oxygenic photosynthesisers an advantage over all other forms of life ...*

*There are six known pathways for fixing atmospheric carbon, of which the Calvin Cycle used in oxygenic phototrophs is the least efficient in terms of the energy and the reducing equivalents (electrons)required per mole of fixed CO₂. Rubisco has a very low turnover for fixing carbon, and its carboxylase activity is compromised by opposing oxygenase activity that uses molecular oxygen to break down Ribulose-1,5-bisphosphate rather than fix CO₂ into it. Despite this, the first inventor of water-splitting was successful, and filled the niche ...*

*Oxygenesis evolved only once. There are two possible explanations for this. One is that it is a Random Walk process, requiring a sequence of unlikely evolutionary steps, which would not have evolved elsewhere. The hypotheses on the origins of oxygenesis above hint this may not be the case, but do not prove it. The other explanation is that the evolution of oxygenesis is a Many Paths process, one which has a high probability of occurring, but is also a Pulling Up the Ladder event, such that once oxygenesis evolved once that evolution removed the preconditions for its evolution again, in this case filling the niche of a photosynthesiser freed from limitation of an electron donor supply. The biochemistry of oxygenic photosynthesis points toward this second explanation.*

Bandfield, J.L., Glotch, T.D*least tens of thousands of years ... and possibly for hundreds of millions of years*

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*These small, spherical, lipid membrane-bound structures typically range in size from ~20 to 200 nm diameter and provide a means for cells to interact with their environment over both spatial and temporal scales*

*Perhaps one of the most striking features of extracellular vesicles is that they can contain nucleic acids (Dorward et al., 1989; Valadi et al., 2007; Rumbo et al., 2011; Biller et al., 2014). DNA fragments of diverse sizes, ranging from hundreds of bp to >20 kb have been reported in vesicles from Gram-negative bacteria, Gram-positive bacteria, archaea and eukaryotes, and include genomic, plasmid and viral DNA (Dorward and Garon, 1990; Klieve et al., 2005; Soler et al., 2008; Biller et al., 2014; Gaudin et al., 2014; Jiang et al., 2014; Grande et al., 2015; Yáñez-Mó et al., 2015). As such, vesicles can function as vehicles of horizontal gene exchange (Yaron et al., 2000; Renelli et al., 2004; Klieve et al., 2005). Shotgun sequencing of vesicle-associated DNA from ocean samples has revealed sequences from diverse bacteria, archaea and eukaryotes (Biller et al., 2014), suggesting that vesicles could be an important mechanism mediating gene transfer among marine microbes.*

Billi, D., Staibano, C., Verseux, C., Fagliarone, C., Mosca, C., Baqué, M., Rabbow, E. and Rettberg, P., 2019a. [Dried biofilms of desert strains of Chroococcidiopsis survived prolonged exposure to space and Mars-like conditions in low Earth orbit](https://www.researchgate.net/profile/Cyprien-Verseux/publication/331027480_Dried_Biofilms_of_Desert_Strains_of_Chroococcidiopsis_Survived_Prolonged_Exposure_to_Space_and_Mars-like_Conditions_in_Low_Earth_Orbit/links/5ee9e56d299bf1faac5c948f/Dried-Biofilms-of-Desert-Strains-of-Chroococcidiopsis-Survived-Prolonged-Exposure-to-Space-and-Mars-like-Conditions-in-Low-Earth-Orbit.pdf). Astrobiology, 19(8), pp.1008-1017.

*Our results suggest that bacteria might indeed survive on Mars if shielded from UV, for instance by martian dust, since it is known that a few millimeters of soil is enough for UV protection (Mancinelli and Klovstad, 2000; Cockell and Raven, 2004). In view of the resistance of desert strain of Chroococcidiopsis to ionizing radiation (Billi et al., 2000; Verseux et al., 2017), the exposure in LEO to a total dose of 0.5 Gy of ionizing radiation did not affect biofilm survival. Hence, based on the dose of 76 mGy/year measured by the Curiosity rover at Gale Crater’s surface (Hassler et al., 2013), dried biofilms would survive on Mars more than half a decade. In addition, since the UV dose received in LEO corresponds to approximately 8 h under a Mars UV flux at the equator (Cockell et al., 2000), the speculated biofilm survival supports the possible dissemination of viable organisms. If carried, for instance, by winds at 5 m/sec (Gomez-Elvira et al., 2014) with the average flux mentioned above, they could travel more than 100km without dying. However, other factors found on Mars need to be taken into account so as to reduce the planetary protection risk, such as the presence of perchlorates that have been shown to be highly damaging to life (Wadsworth and Cockell, 2017)*

Billi, D., Verseux, C., Fagliarone, C., Napoli, A., Baqué, M. and de Vera, J.P., 2019b. [A desert cyanobacterium under simulated Mars-like conditions in low Earth orbit: implications for the habitability of Mars](https://www.researchgate.net/profile/Daniela-Billi/publication/331027480_Dried_Biofilms_of_Desert_Strains_of_Chroococcidiopsis_Survived_Prolonged_Exposure_to_Space_and_Mars-like_Conditions_in_Low_Earth_Orbit/links/5ca21364a6fdcc1ab5ba0613/Dried-Biofilms-of-Desert-Strains-of-Chroococcidiopsis-Survived-Prolonged-Exposure-to-Space-and-Mars-like-Conditions-in-Low-Earth-Orbit.pdf). *Astrobiology*, *19*(2), pp.158-169.

*In this experiment, survival of the Chroococcidiopsis strain occurred only with those cells that were mixed with martian regolith simulant and plated as thin layers (about 15–30 μm, corresponding to 4–5 cell layers).*

*… Our finding suggests that a putative microbial life-form at least as resistant to desiccation and radiation as the investigated desert cyanobacterium could withstand some exposure to UV on the martian surface.*

*… Our findings support the hypothesis that opportunistic colonization of protected niches on Mars, such as in fissures, cracks, and microcaves in rocks or soil, could have enabled life to remain viable while being transported to a new habitat*

Billings, L., 2015, Making Space for Everyone: [A Q&A with BoldlyGo's Jon Morse](https://www.scientificamerican.com/article/making-space-for-everyone-a-q-a-with-boldlygo-s-jon-morse), Scientific American

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Board, S.S. and National Research Council, 2009. *Assessment of planetary protection requirements for Mars sample return missions*. National Academies Press. [page 48](https://www.nap.edu/read/12576/chapter/7#48)

[5, Potential for Large Scale Effects](https://nap.nationalacademies.org/read/12576/chapter/7#48)

*"Despite suggestions to the contrary, it is simply not possible, on the basis of current knowledge, to determine whether viable Martian life forms have already been delivered to Earth. Certainly in the modern era, there is no evidence for large-scale or other negative effects that are attributable to the frequent deliveries to Earth of essentially unaltered Martian rocks. However the possibility that such effects occurred in the distant past cannot be discounted.”*

Board, S.S. and National Research Council, 2012. [*Vision and voyages for planetary science in the decade 2013-2022*](https://solarsystem.nasa.gov/resources/598/vision-and-voyages-for-planetary-science-in-the-decade-2013-2022/). National Academies Press.

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***“Revised Finding 3-1:*** *Cell division by Earth microbes has not been reported below –18°C (255K). The very low rate of metabolic reactions at low temperature result in doubling times ranging from several months to year(s). Current experiments have not been conducted on sufficiently long timescales to study extremely slow-growing microorganisms.”*

Board, S.S., 2019. [Planetary protection classification of sample return missions from the Martian moons](https://nap.nationalacademies.org/catalog/25357/planetary-protection-classification-of-sample-return-missions-from-the-martian-moons), European Space Sciences Committee and National Academies of Sciences, Engineering, and Medicine

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*Kasting: “After doing some rough calculations on the effects of a mirror cyanobacteria invasion, Jim Kasting isn’t sure which would kill us first—the global famine or the ice age. “It would quickly consume all the available nutrients,” he says. “This would leave fewer or perhaps no nutrients for normal organisms.” That would wipe out the global ocean ecology and starve a significant portion of the human population. As the CO₂ in the ocean was incorporated into inedible mirror cells, they would “draw down” CO₂ from the atmosphere, Kasting says. For a decade or two, you would have a cure for global warming. But Kasting predicts that in about 300 years the bugs would suck down half of Earth’s atmospheric CO₂. Photosynthesis of most land plants would fail. “All agricultural crops other than corn and sugar cane would die,” he says. (They do photosynthesis a little differently.) “People might be able to subsist for a few hundred years, but things would be getting pretty grim much more quickly than that.” After 600 years, we’d be in the midst of a global ice age. It would be a total evolutionary reboot—both Kasting and Church think mirror predators would evolve, but whatever life existed on Earth by that point wouldn’t include us..*

Bontemps, J, 2015, [Follow up: Signs of Ancient Life in Mars Rover Photos?](http://www.astrobio.net/mars/follow-signs-ancient-life-mars-rover-photos/) NASA Astrobiology Magazine

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*802: Future missions would therefore benefit from the development of instruments capable of direct and unambiguous detection of extant life in situ, and improvements are needed in capabilities for sample preparation to optimize biosignature detection. Spacecraft resources should support a sufficient number of sample analyses to support replicate analyses, positive and negative controls. Contamination control should be coupled with contamination knowledge so that Earth-sourced material can be eliminated as a possible source of any biological material discovered in Martian samples.*

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*From the perspective of planetary protection, Conley is also concerned about terrestrial organisms that can absorb water from the air. She recalls fieldwork she did in the Atacama Desert in Chile, which is one of the driest places on Earth, with less than 0.04 inch of rain a year.*

*Even in this dessicated place, she found life: photosynthetic bacteria that had made a home in tiny chambers within halite salt crystals. There’s a small amount of water retained inside the halite and, at night, it cools down and condenses both on the walls of the chambers and on the surface of the organisms that are sitting there.*

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***Section 3.3:*** *In the Moon’s formation temperatures of proto-Earth and progenitor impactor material in simulations grow to thousands of Kelvins, sufficient to drive off the great majority of all volatiles, but these are not necessarily the only masses in the system. Either body might have been orbited by satellites containing appreciable volatiles, which would likely not be heated to a great degree and which would have had a significant probability of being incorporated into the final moon. Furthermore, there is recent discussion of significant water being delivered to Earth/Moon distances from the Sun in the minerals themselves (Lunine et al. 2007, Drake & Stimpfl 2007), and these remaining mineral-bound even at high temperatures up to 1000K (Stimpfl et al. 2007). The volume of surface water on Earth is at least 1.4 × 109 km3, so even if the specific abundance of lunar water is depleted to 10−6 terrestrial, one should still expect over 1010 tonnes endogenous to the Moon, and it is unclear that later differentiation would eliminate this. This residual quantity of water would be more than sufficient to concern us with the regolith seepage processes outlined above.*

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[Dr David Williams](http://www.nhm.ac.uk/our-science/departments-and-staff/staff-directory/david-williams.html), a Researcher of Diatoms at the Museum, says 'Yes, technically tiny life forms such as diatoms and cyanobacteria could survive in these environments. But that is not the question we should be asking.

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*Improved instrumentation on rovers that might detect and identify a diversity of potential in situ biosignatures, including ancient organic molecular biosignatures, designed with the ability to differentiate biotic and abiotic signals in micro- or macrostructures. Instrumentation could also be better attuned to the unique complications of biosignature preservation on Mars (e.g., deeper drilling to access potentially better preserved organics)*

*The fluorescence spectrometers on SHERLOC can detect condensed carbon and aromatic organics by deep UV-induced fluorescence, and SHERLOC's Raman spectrometer will allow classification of aromatic and aliphatic organics. Raman spectrometry can also be used to detect minerals relevant to aqueous chemistry. While these measurements would allow us to identify reduced carbon compounds, there may not be sufficient structural information to distinguish between a biological signal and extraterrestrial organic input.*

*A major knowledge gap that will directly impact our ability to choose an appropriate landing site is what terrestrial analog environments might look like—what the biosignature signals might be—if photosynthetic microorganisms had not evolved and instead the environments were only inhabited by chemosynthetic microorganisms*

*4.4. Strategies and priorities*

*In many of the environments discussed, there is a dichotomy between habitability and preservation—many of the conditions that make an environment more habitable are destructive to one or more of the biosignatures of interest. For example, fluid flow in the subsurface of hydrothermal environments helps create the redox gradients that support communities that inhabit the outflow channel. Fluids are also essential for lithification and the associated decrease in permeability essential for long-term preservation. Preservation is enhanced by rapid burial and mineral precipitation that encases and lithifies biological materials in less permeable matrices—in these cases, silica from hydrothermal environments, or silica-enriched aqueous environments, is an important material for preservation. However, these same fluids can degrade biosignatures such as mineralogy, chemistry, and micro- and macrostructures. One strategy for astrobiological exploration has to be to seek out a “sweet spot” where these two balance each other so that long-term preservation is possible. This sweet spot may occur as conditions change through time.*

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*As shown in Table 2, when a sample containing 100,000 spores was analyzed, either Bleach Rite® or 10% bleach was able to dramatically reduce (<0.0001% remaining) the number of viable spores at the earliest time point, and no viable spores were detected after 20 minutes of treatment. Complete sterilization was not attained until 20 minutes post-inoculation due to 1 cfu being present at 10 minutes in the 10% bleach-treated groups.*

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With consideration of upcoming Mars-targeted missions, we conclude that gas collected in a newly designed and purpose-built valved sample-tube sized vessel, which could be flown on either SFR or SRL, would be considered of higher priority than either the head space gas or a sealed M2020 sample tube. Conceptually, this vessel would require no more physical space to return than a sealed empty sample tube and alleviate concerns about the manufacturing and history of a non-purpose-built vessel, and the valving would provide a more robust mechanism for sealing the vessel and testing the seal upon return.

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*On Spirit there is a yearly pattern with steady dust deposition throughout roughly the colder half year from late southern summer to late southern winter, which encompasses the Martian aphelion, and overall dust removal during the warmer and windier perihelion season from late southern winter to late southern summer.*

*On Opportunity ... the overall variation between highs and lows is smaller, and there are two periods of overall dust deposition and two periods of overall dust removal every year. The deposited dust thickness peaks once in the middle of the northern hemisphere spring. This peak recurs very regularly 6 times. ... There is also a peak roughly in the middle of the southern spring. This peak is clear in the first year, but the pattern becomes more irregular later in the mission and is entirely absent in the last year.*

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**LM:** Pamela and I disagree on this, but there’s a provision in the Outer Space Treaty, Article VI, which says that each country must supervise and authorize the activities of its nongovernmental entities. This is not a self-executing provision, and the U.S. Supreme Court has held that a non-self-executing treaty is not domestically enforceable. ...

**PM:** I disagree with Laura on this. Article VI of the Outer Space Treaty provides that all state parties to the treaty are responsible for their activities in outer space, whether they’re carried out by government agencies or private companies. Countries are required to subject private companies within their jurisdiction that engage in space activities to an authorization requirement and continuing supervision. So, the United States is responsible for compliance with the Outer Space Treaty by our private companies or entities that go into space.

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*The desiccated bacteria are constituents of the dust occasionally blown up by the winds into the atmosphere and stratosphere. where bacteria from different geographic origins mix while being exposed to UVC light 100 to 1000 times more intense than on Earth’s surface. They eventually rehydrate when falling back on Earth with the rain and snow (this is how Francois-Xavier Pellay in our laboratory collects robust bacteria) and—depending on their genomic constitution—develop, or not, in the ecological niches into which they happen to fall. Indeed, the most efficient cellulose degraders are deinococci found growing in the waist of the wood-sawing industry (Deinove, pers. comm.).*

*The resistance of D. radiodurans is not exclusive to radiation and desiccation but extends also to many toxic chemicals and conditions. Therefore, Dra is called a polyextremophile, a robust “generalist,” to be distinguished from specialized extremophiles with an evolutionary redesign of their proteome (e.g., proteins purified from thermophiles are thermostable in vitro). Unlike specialized extremophiles, Dra does not thrive on extreme conditions—indeed, it does not grow while desiccated or when heavily* irradiated—but it can reproduce under standard growth conditions after recovering from damage inflicted by chronic moderate, or acute intense, exposures to cytotoxic conditions.

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my visit to you November 6, 1957….*

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*“We have discovered the substance calcium perchlorate in the soil and, under the right conditions, it absorbs water vapour from the atmosphere. Our measurements from the Curiosity rover’s weather monitoring station show that these conditions exist at night and just after sunrise in the winter. Based on measurements of humidity and the temperature at a height of 1.6 meters and at the surface of the planet, we can estimate the amount of water that is absorbed. When night falls, some of the water vapour in the atmosphere condenses on the planet surface as frost, but calcium perchlorate is very absorbent and it forms a brine with the water, so the freezing point is lowered and the frost can turn into a liquid. The soil is porous, so what we are seeing is that the water seeps down through the soil. Over time, other salts may also dissolve in the soil and now that they are liquid, they can move and precipitate elsewhere under the surface,” explains Morten Bo Madsen, associate professor and head of the Mars Group at the Niels Bohr Institute at the University of Copenhagen.*

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*(Admittedly there is uncertainty concerning the delay between change in temperature at the head end assembly, perhaps one inch over the 0.5 cc soil sample, and soil sample temperature per se. However, a two-hour lag seems quite long for what is presumably a convective and radiative process. Similarly, thermal-induced movement of gas between the soil sample and the beta detector requires only about 20 minutes.)*

*Furthermore, the LR oscillation does not slavishly follow the thermal variation; rather, it seems that the LR rhythm is extracted from the HT oscillation, while high frequency noise is not. This is very common in terrestrial organisms in which a low frequency periodic stimulus (i.e., a zeitgeber) such as a 12:12 light/dark cycle can entrain a circadian rhythm, while high frequency transients in the same stimulus are ignored (e.g., turning on the light in the bathroom at night for a minute or two does not alter normal entrainment to the light/dark cycle).*

*Furthermore, there is abundant evidence that as little as a 2º C temperature cycle can entrain circadian rhythms in terrestrial organisms such as lizards, fruit flies, and bread molds and entrainment can be preferential to the diminution phase of the temperature cycle, in analogy to the temperature fall that occurs at sunset on Mars).*

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*After 7 years of air-drying, Chroococcidiopsis not only avoided genome degradation but preserved at least a sub-set of mRNAs and 16S ribosomal RNA.*

*... In the present work, the occurrence of survivors in dried biofilms and dried-UV-irradiated biofilms was proved by growth after transfer into liquid BG-11 medium (not shown) and by INT reduction after 72 h of rewetting.*

*Reshaping the boundaries of Chroococcidiopsis desiccation and UV tolerance has implications in the search for extra-terrestrial life since it contributes to defining the habitability of Mars and planets orbiting other stars. In fact, the UV dose used here corresponds to that of a few hours at Mars’s equator (Cockell et al., 2000). Hence, considering that survivors occurred in the bottom layers of the biofilms (Baqué et al., 2013), it might be hypothesized that if a biofilm life form ever appeared during Mars’s climatic history, it might have been transported in a dried state under UV radiation, from niches that had become unfavorable to niches that were inhabitable (Westall et al., 2013). The reported survival also suggests that intense UV radiation fluxes would not prevent the presence of phototrophic biofilms or their colonizing of the landmass of other planets.*

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"*It has been estimated that the planning, design, site selection, environmental reviews, approvals, construction, commissioning, and pre-testing of a proposed SRF will occur 7 to 10 years before actual operations begin. In addition, 5 to 6 years will likely be required for refinement and maturation of SRF-associated technologies for safely containing and handling samples to avoid contamination and to further develop and refine biohazard-test protocols. Many of the capabilities and technologies will either be entirely new or will be required to meet the unusual challenges of integration into an overall (end-to-end) Mars sample return program.*"

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2.5 [Cost Effectiveness considerations](https://www.nasa.gov/seh/2-5-cost-effectiveness-considerations#Fig2-5-1https://www.nasa.gov/seh/2-5-cost-effectiveness-considerations)

3.3 [Project Pre-Phase A: Concept Studies](https://www.nasa.gov/seh/3-3-project-pre-phase-a-concept-studies)

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A picture containing timeline

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*page 15: The NC-77 transgenic line, however, had a three-fold increase in bio-mass yield compared with wild-type. This increased bio-mass production in NC transgenics with adjustable light harvesting antenna sizes, however, raises the question why have algae and plants evolved large, less effi-cient, fixed light-harvesting antenna systems that oversaturate downstream electron transfer processes during most (80%) of the day. In mixed species environments, the abil-ity to shade or reduce the light available to competing spe-cies may offer a selective advantage, because limiting light availability to other species would reduce their growth rates and presumably their fitness (Zhuet al., 2008; Ortet al., 2015). Species competing for light are clearly impacted by shading as plant canopies close or as algal cultures reach high cell densities. Thus, having large light-harvesting antenna systems may reduce light availability for competitors and enhance fitness for plants or algae thatshade competitors as is the case in high-density algal cul-tures. In addition, plants living lower in the canopy or algae growing deeper in the water column often experi-ence very low light conditions.*

*Having a large light-harvesting antenna would allow photosynthesis and growth at light intensities that could not support the growth of algae with smaller antenna sizes optimized for growth at higher light intensities. In fact, algae that grow at extreme depths in the oceans have among the largest light-harvesting antenna sizes known in photosynthetic organisms (Yamazakiet al., 2005).*

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A critical nutrient to the expansion of both subsurface and surface life on any planet is the availability of nitrogen as an aqueous species. On Earth, microorganisms evolved the ability to fix N2 into ammonia with the development of nitrogenase to overcome this constraint. Nitrogenases, Nif proteins, are complex enzymes, utilizing iron, molybdenum, and/or vanadium, that exist in both bacterial and archaeal domains. Phylogenetic comparison of genes that comprise nitrogenases and a complement of proteins required for their regulation indicate that nitrogenases emerged in anoxic sulfidic environments on Earth within obligate anaerobic thermophilic methanogens and were transferred to obligate anaerobic clostridia (Boyd et al., 2015), both common subsurface microorganisms. As Nif proteins were adopted first by the aerobic diazotrophic lineage Actinobacteria and then by the more recently evolved aerobic Proteobacterial and Cyanobacterial lineages, the Nif protein suite became more complex to protect the core MoFe-bearing proteins from O2 (Boyd et al., 2015). Although it is not clear whether the emergence of the more complex protein occurred prior to or after the Great Oxidation Event, it is certain that the ancestral protein emerged in an anoxic environment when the demands for aqueous nitrogen species exceeded the abiotic supply. The implications for martian ecosystems are that nitrogenase would have also likely emerged within an anaerobic subsurface environment, not in the oxic surface environment.

Experiments on the effects of low pN2 on diazotrophic nitrogen-fixing soil bacteria have shown that they could grow in N2 partial pressures of 5 mbar but not 1 mbar (Klingler et al., 1989). This result suggests that further experiments on wild-type species are required to determine whether the evolution of pN2 in the martian atmosphere was a significant deterrent to the expansion of early life, especially after Mars lost most of its atmosphere. Analyses of the nitrogen budget and of nitrogen cycling from deep subsurface environments in South Africa indicate that the pN2 is higher at depth than on the surface, that most of this N2 originates from the rock formations through nitrogen cycling, and that N2 is being actively fixed in the subsurface by microbial communities (Silver et al., 2012; Lau et al., 2016b). Given the presence of a cryosphere barrier to diffusion on Mars, the nitrogen availability and perhaps even the pN2 of subsurface brines are likely to be higher there than on the martian surface.

…

*Meter-sized Fe(II)-rich carbonate/iron oxide concretions (Fig. 4) are found in Jurassic sandstone deposits of southwest Colorado that were formed at hundreds of meters' depth between 2 and 0.5 Ma as the Colorado River Basin was uplifted (McBride et al., 2003; Loope et al., 2010). Similar-sized ferroan calcite and siderite concretions occur in Late Paleocene/Early Eocene Wasatch Group sandstones, and siderite nodule-bearing cores from the formation (Lorenz et al., 1996) yielded thermophilic Fe(III)-reducing bacteria that were capable of producing prodigious quantities of siderite (Roh et al., 2002). In subaqueous systems unconstrained by rock matrix, authigenic carbonate mounds at CH4 and hydrocarbon seeps, formed from carbon mobilized by methane- and alkane-oxidizing microorganisms (Greinert et al., 2001; Formolo et al., 2004; Ussler and Paull, 2008), can be hundreds of meters tall and more than a kilometer wide (Klaucke et al., 2008).*

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*page 8530: A principal limitation of efficient photosynthesis is that organisms absorb more light in full sunlight than they can use productively. The reason seems clear: high absorptivity provides effective capture at low light intensities, such as at dawn and dusk and on cloudy days, and it obviates competition from other phototrophs by absorbing the light before they do.*

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193:

*Meteorite analysis has detected boron in Martian clays, important for abiogenesis since borate minerals can stabilize ribose and catalyze other prebiotic chemistry reactions (see Stephenson et al. 2013 and sources therein). Mars may also have enjoyed greater availability of prebiotically important phosphate than Earth (Adcock et al. 2013). Climate models suggest liquid water was transient on Mars (Wordsworth et al. 2013b), which suggests the evidence of wet/dry cycles. Such cycles are useful for prebiotic chemistry: aqueous eras are beneficial for the formation of biotic monomers, while dry eras tend to concentrate feedstock molecules and aid monomer polymerization (Benner & Kim 2015), relevant to the formation of nucleotides and amino acids (Patel et al. 2015). Finally, the putative dryness of Mars and the potential acidity of its early aqueous environment owing to dissolved carbonic acid from a CO₂ -dominated atmosphere, suggest molybdate, which is suggested to catalyze formation of prebiotically important sugars such as ribose, may have been stable on Mars (Benner & Kim 2015; Benner et al. 2010). Hence, there is growing interest in the possibility that prebiotically important molecules may have been produced on Mars (Benner 2013), and even the hypothesis that life may have originated on Mars and been seeded to Earth (Kirschvink & Weiss 2002; Gollihar et al. 2014; Benner & Kim 2015)*

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Contains:

* ***A Case for Caution*** by John Rummel, NASA'S planetary protection officer at the time, and previously, NASA senior scientist for Astrobiology
* ***Hazardous Until Proven Otherwise***, by Margaret Race, a biologist working on planetary protection and Mars sample return for the SETI Institute and specialist in environment impact analysis
* ***Practical Safe Science*** by Kenneth Nealson, Director of the Center of Life Detection at NASA's JPL at the time.

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*Pages 94-5: Questions about the adequacy of the SRF to maintain the new life form must also be addressed, including the possible need to add equipment, change operations, review emergency plans, or upgrade the facilities because of what has been found.*

*Concerns about security should also be reconsidered, especially in view of the potential disruptive activities of any terrorists or ‘radical’ groups that may be opposed to sample return. The advisability of allowing distribution of untested sample material outside the SRF2684* may need to be reconsidered, as well.

*Plans should be developed well in advance in order to avoid a frenzied, reactive mode of communications between government officials, the scientific community, the mass media, and the public. Any plan that is developed should avoid a NASA-centric focus by including linkages with other government agencies, international partners, and external organizations, as appropriate. It will also be advisable to anticipate the kinds of questions the public might ask, and to disclose information early and often to address their concerns, whether scientific or non-scientific.  
...*

*Evaluations of the proposal should be conducted both internal and external to NASA and Centre National d’Etudes Spatiale (CNES) and the space research communities in the nations participating in the mission. An ethical review should be conducted at least at the level of the Agencies participating and these reviews made public early in the process (in France, the national bioethics committee, Comité Consultatif National d'Ethique pour les Sciences de la Vie et de la Santé, CCNE, is the appropriate organization). The final protocol should be announced broadly to the scientific community with a request for comments and input from scientific societies and other interested organizations. Broad acceptance at both lay public and scientific levels is essential to the overall success of this research effort.*

In the long term, the discovery of extraterrestrial life, whether extant or extinct, in situ or within returned sample materials, will also have implications beyond science and the SRF per se. Such a discovery would likely trigger a review of sample return missions, and plans for both robotic and human missions. Legal questions could arise about ownership of the data, or of the entity itself, potentially compounded by differences in laws between the United States and the countries of international partners. In any event, ethical, legal and social issues should be considered seriously. Expertise in these areas should be reflected in the membership on appropriate oversight committee(s).

Page 101: **Communications** Unusual or unprecedented scientific activities are often subject to extreme scrutiny at both the scientific and political levels. Therefore, a communication plan must be developed as early as possible to ensure timely, and accurate dissemination of information to the public about the sample return mission, and to address concerns and perceptions about associated risks. The communication plan should be pro-active and designed in a manner that allows the public and stakeholders to participate in an open, honest dialogue about all phases of the mission with NASA, policy makers, and international partners. Risk management and planetary protection information should be balanced with education/outreach from the scientific perspective about the anticipated benefits and uncertainties associated with Mars exploration and sample return.

The communication plan should also address how the public and scientific community will be informed of results and findings during Life Detection and Biohazard testing, including the potential discovery of extraterrestrial life. Because of the intense interest likely during initial sample receipt, containment, and testing, procedures and criteria should be developed in advance for determining when and how observations or data may be designated as “results suitable for formal announcement.” Details about the release of SRF information, the management of the communication plan, and its relationship to the overall communications effort of the international Mars exploration program should be decided well in advance of the implementation of this protocol.

Rummel, J.D., Beaty, D.W., Jones, M.A., Bakermans, C., Barlow, N.G., Boston, P.J., Chevrier, V.F., Clark, B.C., de Vera, J.P.P., Gough, R.V. and Hallsworth, J.E., 2014.A [new analysis of Mars “special regions”: findings of the second MEPAG Special Regions Science Analysis Group (SR-SAG2)](https://mepag.jpl.nasa.gov/reports/Rummel_et_al_Astrobiology_14-SR-SAG2.pdf)

Rummel, J. D., Conley C. A, 2017,.[Four fallacies and an oversight: searching for Martian life](http://online.liebertpub.com/doi/full/10.1089/ast.2017.1749) *Astrobiology*, *17*(10), pp. 971-974.

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## S

Sagan, C, 1961. [Organic matter and the Moon](https://www.nap.edu/download/18476)., National Academy of Sciences.

[*Page 23*](https://www.nap.edu/read/18476/chapter/5#23)*: It is remarkable that the depth at which surviving lunar organic matter is expected to be localized (section II) is just the depth at which temperatures appear to be optimum for familiar organisms (section IV). At such temperatures and depths, some moisture should be expected, arising from meteoritic and organic bound water. Watson, Murray and Brown (1961) have recently pointed out that ice could have been retained on permanently shaded areas of the Moon. These circumstances provide all the survival requirements of many terrestrial organisms - water and their metabolites, appropriate temperature, and negligible radiation. That autochthons evolving with the changing environment could also survive under these conditions is far from inconceivable.*

Sagan, C., Levinthal, E.C. and Lederberg, J., 1968. [Contamination of Mars](https://profiles.nlm.nih.gov/ps/access/BBABJH.ocr). *Science*, *159*(3820), pp.1191-1196.

*"The prominent dust storms and high wind velocities previously referred to imply that aerial transport of contaminants will occur on Mars. While it is probably true that a single unshielded terrestrial microorganism on the Martian surface ... would rapidly be enervated and killed by the ultraviolet flux, ... The Martian surface material certainly contains a substantial fraction of ferric oxides, which are extremely strongly absorbing in the near ultraviolet. ... A terrestrial microorganism imbedded in such a particle can be shielded from ultraviolet light and still be transported about the planet."*

*…*

*"A single terrestrial microorganism reproducing as slowly as once a month on Mars would, in the absence of other ecological limitations, result in less than a decade in a microbial population of the Martian soil comparable to that of the Earth's. This is an example of heuristic interest only, but it does indicate that the errors in problems of planetary contamination may be extremely serious."*

Sagan, C., 1973, [*The Cosmic Connection - an Extraterrestrial Perspective*](https://www.e-reading.life/bookreader.php/148581/Sagan_-_The_Cosmic_Connection___An_Extraterrestrial_Perspective.pdf)

*I reach this conclusion reluctantly. I, myself, would love to be involved in the first manned expedition to Mars. But an exhaustive program of unmanned biological exploration of Mars is necessary first****. The likelihood that such pathogens exist is probably small, but we cannot take even a small risk with a billion lives.*** *Nevertheless, I believe that people will be treading the Martian surface near the beginning of the twenty-first century.*

Sagan, C., 1977. [Reducing greenhouses and the temperature history of Earth and Mars](https://www.nature.com/articles/269224a0). *Nature*, *269*(5625), pp.224-226.

Sagan, C., 1980., *Cosmos: The Story of Cosmic Evolution, Science and Civilisation*

full quote:

*The surface area of Mars is exactly as large as the land area of the Earth. A thorough reconnaissance will clearly occupy us for centuries. But there will be a time when Mars is all explored; a time after robot aircraft have mapped it from aloft, a time after rovers have combed the surface, a time after samples have been returned safely to Earth, a time after human beings have walked the sands of Mars. What then? What shall we do with Mars?*

*There are so many examples of human misuse of the Earth that even phrasing this question chills me. If there is life on Mars, I believe we should do nothing with Mars. Mars then belongs to the Martians, even if the Martians are only microbes. The existence of an independent biology on a nearby planet is a treasure beyond assessing, and the preservation of that life must, I think, supersede any other possible use of Mars.*

Sagan, C., 1997. [Pale blue dot: A vision of the human future in space](http://www.planetary.org/explore/space-topics/earth/pale-blue-dot.html). Random House Digital, Inc..

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*page 37: Normal wild-type algae have large chlorophyll-bindingLHCII antenna systems and consequently the culture is dark green. Cell lines with small LHCII antenna systems yield cultures which are a much lighter green at the same cell density (Fig.7a). In the wild-type case, algal cells at the illuminated surface of the bioreactor that are exposed to high light levels capture the bulk of the light, but waste upto∼90% of the energy as fluorescence and heat [122,134].*

*As a result the wild-type cells located deeper in the culture are exposed to ever decreasing levels of light the further they are from the illuminated surface (see“Open PondSystems”section). These shaded cells are prevented from capturing enough solar energy to drive photosynthesis efficiently. This in turn drastically reduces the efficiency of the overall culture.In contrast, small antenna cell lines with reduced LHCIIlevels have the advantage that they improve the light penetration into the bioreactor (Fig.7a) and better match itto the energy requirements of each photosynthesizing cell. Thus small antenna cells at the bioreactor surface absorb only the light that they need, largely eliminating fluores-cence of excess energy. This in turn allows more light (i.e.the light wasted in wild-type as fluorescence and heat) to penetrate into the bioreactor so that even cells deeper in the culture have a near optimal exposure to light*

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*Historically the species was restricted to low-nutrient waters but has recently seen large range expansions reportedly occurring in eutrophic rivers, showing much greater tolerance for nutrient and flow conditions than previously expected. This may be attributed to a genetic variant with broader tolerances than the original species.*

***Means of Introduction:*** *Didymosphenia geminata has been shown to survive outside of the stream environment. Cells are able to survive and remain viable for 40 days in cool, dark, damp conditions. Angling equipment, boot tops, neoprene waders, and felt-soles provide a particularly suitable environment for cells to remain viable. Cells can hitchhike on this equipment and other recreational equipment into new bodies of water (Spaulding and Elwell 2007). Freshwater diatoms are dispersed through the flow of water and transport by other organisms, primarily waterfowl (Kristiansen 1996).*

*Blooms of Didymosphenia geminata form mats which can be over 20 cm thick. Extracellular stalks trap fine sediment, changing the nature of substrate and have potential long lasting effects due to the apparent resistance of stalks to degradation by bacteria and fungi*

*…*

*These mats are capable of engulfing the stream bottom, smothering native species of plants, insects, mollusks, and algae, and reducing habitat for insects for aquatic insects and fis*

*Streams outside the Great Lakes region harshly impacted by these mats have seen invertebrate populations decrease, macrophyte elimination, and absence of fish*

*It has been hypothesized that a new strain of Didymosphenia geminata is now dominant (Bothwell et al., 2006), and is responsible for the invasive behaviour. However, the presence of a new genetic strain has not been established….*

*Some clusters of dozens of diatoms appear pristine, suggesting that they had been living in the salar pool immediately*

*before being trapped as the gypsum crystal grew.*

*Could microfossils and/or viable microorganisms be trapped in gypsum on Mars as they are in gypsum on Earth? It is likely that abundant sulfate sand grains on Mars contain fluid inclusions similar to those in the acid-precipitated bottom-growth and reworked gypsum we discuss here.*

*We suggest that gypsum on Mars would have entrapped, as solid inclusions and within fluid inclusions, any microorganisms and/or organic compounds that were present in its parent waters. Therefore, fluid inclusions and solid inclusions hosted by salt minerals may be the best place to continue the search for life on Mars.*

*Some of these entrapped microorganisms remain viable for at least tens of thousands of years ... and possibly for hundreds of millions of years*

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***(2) Collecting gas in a newly-designed, valved, sample-tube-sized vessel that is flown on either the Sample Fetch Rover (SFR) or the Sample Retrieval Lander (SRL)***

***...***

***The triple oxygen isotope composition of atmospheric CO2, O2, H2O, and CO would provide a unique picture of Martian atmospheric photochemistry and allow an understanding of the anomalous signatures in Martian minerals and water.***

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*Surprisingly, however, we have found that O₂ does not demonstrate the predictable seasonal behavior of the other major components. Surface O₂ measurements by SAM yield abundances that vary between 1300 and 2200 ppmv; when corrected for the annual global mean pressure, O₂ varies from 1300 to 1900 ppmv. Despite large instrument backgrounds, these are the first precise in situ measurements of O2, revealing a surprising seasonal and interannual variation that cannot be accounted for in current chemical models. Though Mars has the potential to generate significant O₂ release due to abundances of oxidants in/at its surface, the mechanisms by which O₂ could be quickly generated and then quickly destroyed are completely unknown. As with all surprising results, we hope that continued in situ, experimental, and theoretical results may shed light on this intriguing observation.*

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