HOW THE COMMON ANCESTRY HYPOTHESIS INFORMS THE SEARCH FOR LIFE ON MARS. C. E. Carr[1] , C. S. Lui[1], M.T. Zuber[1] and G. Ruvkun[2,3], [1]Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, MA, [2]Department of Molecular Biology, Massachusetts General Hospital, Boston, MA , [3]Division of Genetics, Harvard Medical School, Boston, MA.

Introduction: Life on Mars, if it exists, may share a common ancestry with life on Earth derived from meteoritic transfer of microbes between the planets. Martian meteorites were transferred to the Earth at shortened time scales and with higher fluxes than previously believed [1-3]. The final destination of as much as 7.5% of all Martian meteorites is thought to be the Earth, constituting nearly a billion tons of meteoric debris [1], with some elements crossing from one planet to another within thousands of years. Several dozen SNC meteorites of Martian origin have been discovered here on Earth, and magnetic and thermochronological analyses indicate that ~20 weight% of Martian meteorites only experienced mild heating during ejection and impact [4-6]. Recent hypervelocity impact studies confirm that microbes can survive the required shock pressures [7]. Once life had evolved on one of the planets, the rate of material transfer makes it plausible that the adjacent planet could "catch" life instead of independently evolving it. Consistent with this hypothesis, the earliest isotopic evidence for life on Earth closely follows an intense period of meteoritic bombardment known as the late heavy bombardment. The more steps required for biogenesis, the more a Martian origin of life is favored, given this apparent rapid appearance of life on Earth [8].

Many life detection strategies –seeking generic informational polymers, structures of biogenic origin, or chemical or isotopic signatures of enzymatic processes– suffer from lack of specificity due to abiological routes to these signatures, and, in some cases, inadequate sensitivity to find life in lean samples. Here we describe how the shared ancestry hypothesis informs the search for life on Mars and provides a highly sensitive and specific approach to discovering ancestrally related life with an in-situ nucleotide detector.

Nucleotide Detection Strategies: Table 1 summarizes a number of approaches to search for life under the shared ancestry hypothesis. DNA amplification based on the Polymerase Chain Reaction (PCR), which relies upon thermal cycling to exponentially amplify a DNA fragment, is suitable for any of these approaches. Isothermal amplification using ϕ29 polymerase [9-11], is better suited for non-specific amplification using random hexamers, such as for whole genome amplification [12]. A reverse-transcription step can convert RNA into its DNA equivalent for further amplification.

Table 1 – Detection approaches based on shared ancestry

Ancestry Hypothesis	Detection Strategy
A separate genesis on Mars and Earth [13]	DNA detection valid if biochemistry is universal [14]
Transfer during RNA world [15, 16]	Look for RNA using non-specific approach
Transfer after evolution of DNA but before evolution of present-day conserved genes	Look for DNA using non-specific approach such as whole genome amplification and/or random primers
Transfer after evolution of conserved genes such as ribosomal subunits 16S/23S	Look for DNA by targeting highly conserved genes, specifically amplify between highly conserved regions

PCR amplification has been by far the most successful life detection and classification method developed to date. The PCR strategy for life detection on Earth is largely based on targeting sequences within ribosomal RNA (rRNA), genes that have shown lesser variation across the tree of life than most genes. rRNAs are the main structural and catalytic components of the ribosome, a molecular machine that translates RNA into proteins [17]. Within the ~1500 nucleotides of the 16S rRNA gene (18S in eukaryotes), there are multiple 15-20 nucleotide segments that are nearly identical in all known organisms [18]. A 16S sequence captures a huge dynamic range of evolutionary history, with regions of essential catalytic activity changing little even over billions of years (as a consequence of negative selection), and other so-called hyper-variable regions rapidly accumulating inconsequential mutations. Thus, PCR amplification of a sequence spanning two highly conserved regions yields new information about less-conserved regions, providing a powerful genetic signature. Currently, 16S ribosomal RNA gene based PCR is used to prospect for new archaeal and bacterial species from a wide range of environments; thousands of microbial species have been such described. This molecular method led to the definition of entirely new high-level taxa [19-21] and has expanded what we recognize as potential habitable zones.

By using PCR directed at highly conserved genetic regions or general amplification strategies, an in-situ instrument could extract large amounts of information from sequences through analysis of multiple Martian samples. Sequence information from a Mars system of life isolated from Earth life over geologic time will be evident from phylogenic analysis: while rooting the tree of life is complex [22], phylogenetic analysis will reveal whether sequences found on Mars are similar to

those on Earth and likely to represent contamination, or are phylogenetically distant and indicative of extant Martian life that has been isolated from that on Earth for the past 3 billion years.

Targeting conserved genes. All known living organisms share a set of several hundred "universal genes" including 16S [23, 24] and regions of 16S appear to remain intact for billions of years. Targeting these conserved regions may provide us with the strongest evidence of a specific phylogenetic relationship between sequences identified on Mars and Earth life; metagenomic libraries built from specific or non-specific amplification can also be used to build such relationships, and may be mined, for example, for evidence of specific metabolic pathways. Using a diverse set of 26 whole genome sequences we have shown in prior work [25] that sequences within the 16S and 23S genes scored as the most highly conserved sequences, followed by transfer RNAs and ATP binding cassette (ABC) transporter genes. Thus, the 16S and 23S ribosomal RNA genes are the best-conserved elements by which to capture divergent genomes.

Targeting the RNA World. The probable existence of an RNA world that predated the current DNA world was revealed by the discovery in 1989 of ribozymes, RNA based informational molecules that also possess enzymatic activity on RNA [15, 16]. Ribozymes on Earth are living fossils of the RNA world that continue to exist inside modern DNA based life despite the displacement of most of the RNA world. However, if the RNA world was dominant during the period of intense Earth-Mars meteoritic exchange, it is plausible that any Mars RNA world life may not have been displaced by a DNA world, allowing a presumed displaced form of life on Earth to be explored.

Shared ancestry before evolution of conserved genes. The 16S RNA gene may have evolved after the heavy bombardment period on Earth and Mars, or it might have diverged so much that, even if life on Mars was DNA or RNA based and ancestrally related to life on Earth, it would not be detectable using primers targeting 16S and 23S. However, one can also detect ancestrally related DNA- or RNA-based life that does not share these or other conserved genes. One standard method for general DNA amplification is isothermal and utilizes the phage ϕ29 DNA polymerase and random hexamer primers which has already been used to demonstrate whole-genome amplification in microfluidic devices [13, 27] and easily adaptable to amplify and detect nucleic acids in any environmental sample, including on Mars.

Characterization of Detected Nucleotides: A complete discussion of characterization of any Mars "positives" and the potential for contamination is beyond the scope of this paper. However, modern microfluidic and ultra-clean PCR approaches (which yield low false positive and false negative rates at the single molecule amplification threshold), along with careful controls, now make definitive classification of putative Martian organisms feasible. Some of the strategies that can be used to validate positives include: (a) characterize and reduce potential contaminants by metagenomic sequencing of spacecraft clean room samples and treatment of reagent streams, (b) reduce forward contamination through planetary protection and other handling precautions, (c) demonstrate a low false positive rate at the single molecule detection limit using negative controls, (d) require that samples go positive before controls and subsequently classify and sequence those positives, rejecting any sequences highly similar to those in the ever-expanding library of known Earth sequences, (e) search for putative Mars sequences on Earth, and (f) confirm results in multiple samples or sites and coroborate findings with results of other instruments not based on nucleotide detection.

In summary, given the possibility of shared genetic material, nucleotide detection is a critical part of any comprehensive life detection strategy for Mars.

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