Upending Assumptions About Life On Earth

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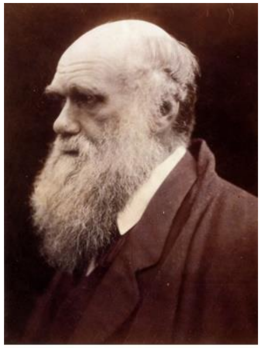
- **Discovery that there really is life in Hell.**
- **Need to study biology under high pressure.**

Paradigm Shift

In 1962 Thomas Kuhn wrote "*The Structure of Scientific Revolutions*": Science proceeds by paradigm shifts.

We are in the midst of a paradigm shift about where much of life on earth exists, what is required to sustain life, and how life evolved.

1871: Charles Darwin letter to J.D. Hooker

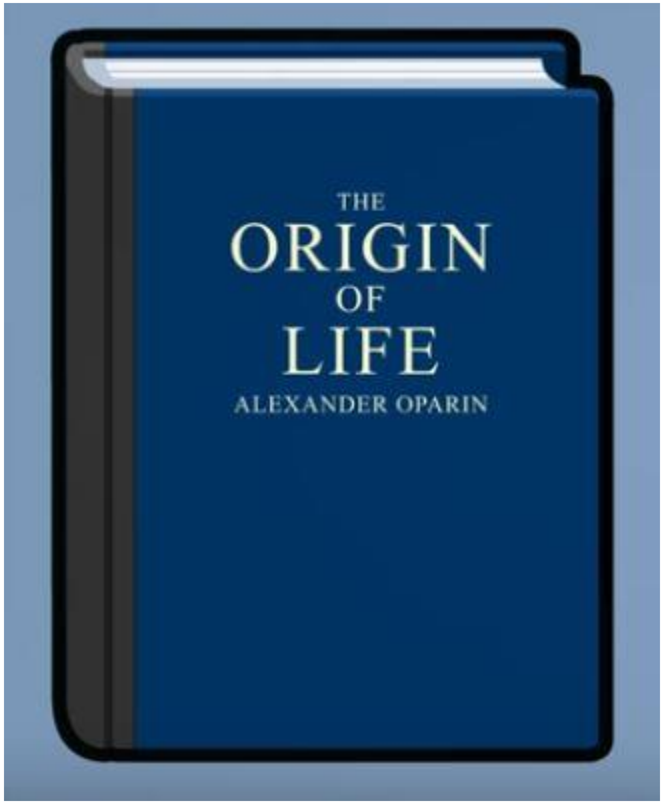


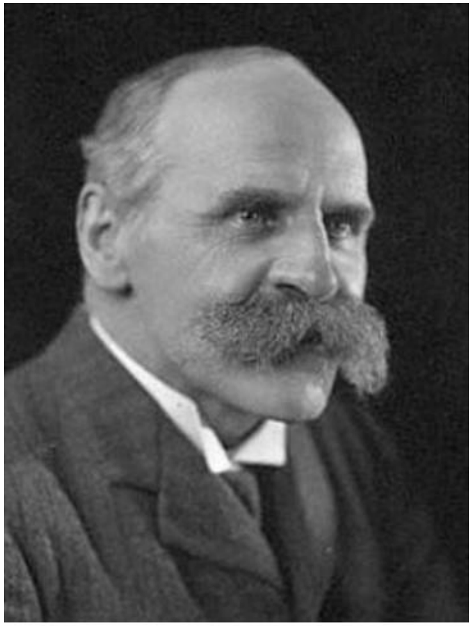
Julia Margaret Cameron, Public domain, via Wikimedia Commons

“But if (& oh what a big if) we could conceive in some warm little pond with all sorts of ammonia & phosphoric salts,—light, heat, electricity &c present, that a protein compound was chemically formed, ready to undergo still more complex changes…”

1920s: Oparin & Haldane
“Primaeval Soup”

Alexander Oparin
1924

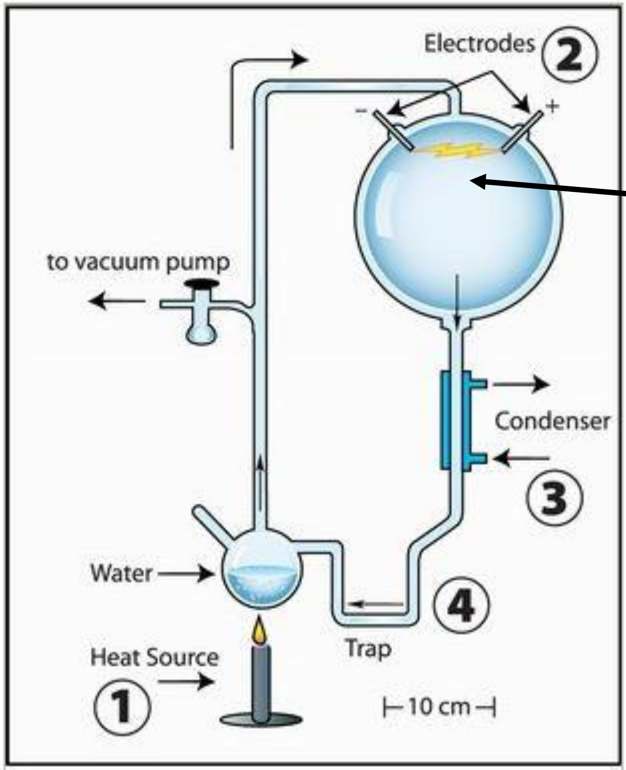




https://en.wikipedia.org/wiki/John_Scott_Haldane

J.B.S. Haldane
1929

1953: Stanley Miller & Harold Urey*



Credit: Ned Shaw, Indiana University

H_2O,
H_2,
NH_3,
CH_4

Recipe for Life

1. **Start with reducing atmosphere**
2. **Expose atmosphere to energy - heat, UV, lightning**
3. **Monomers form – "primordial soup"**
4. **Leading to the large polymers of life: lipids, nucleic acids & proteins.**

*Miller, *Science*, 117 (1953) 528.
See also Garrisson et al., *Science*, 114 (1951) 416

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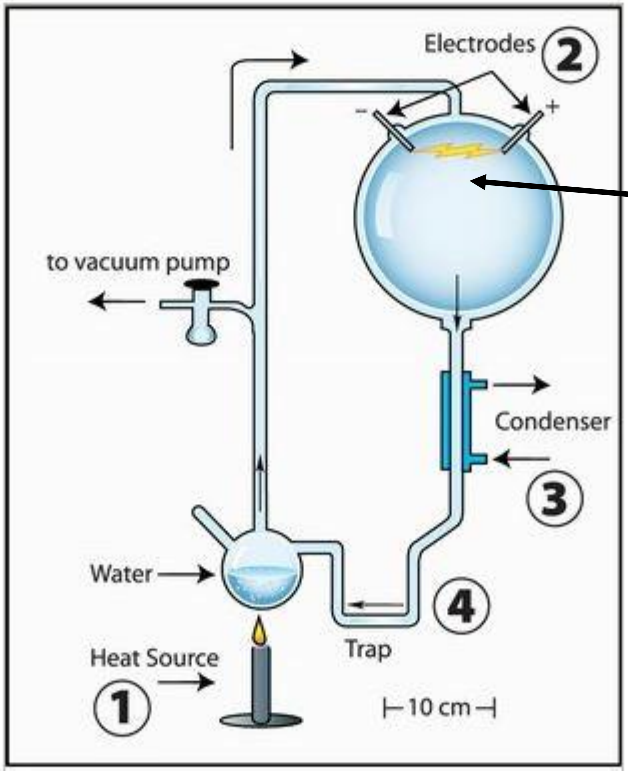

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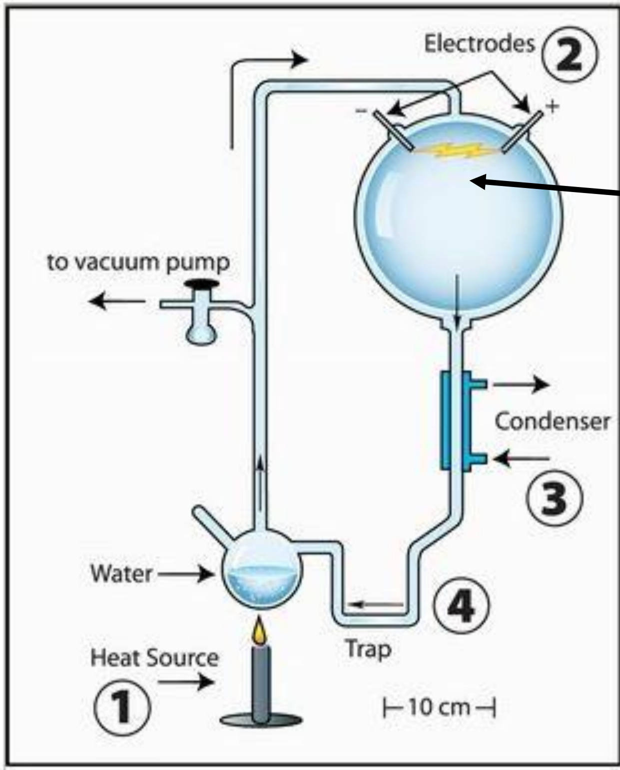

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What is the Biosphere?

- **Up to about 3 decades ago oceans, lakes and land surface were assumed to be the “Biosphere”.**
- **The deep Earth crust was assumed to be sterile:**
  - Without the sun, there is no energy source.
  - The deep crust is a hostile Hi-P, Hi-T place.

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Interest In Deep Life Is Not New

Detailed chronology, see Tullis Onstott, *Deep Life*, (Princeton Univ. Press, 2017)

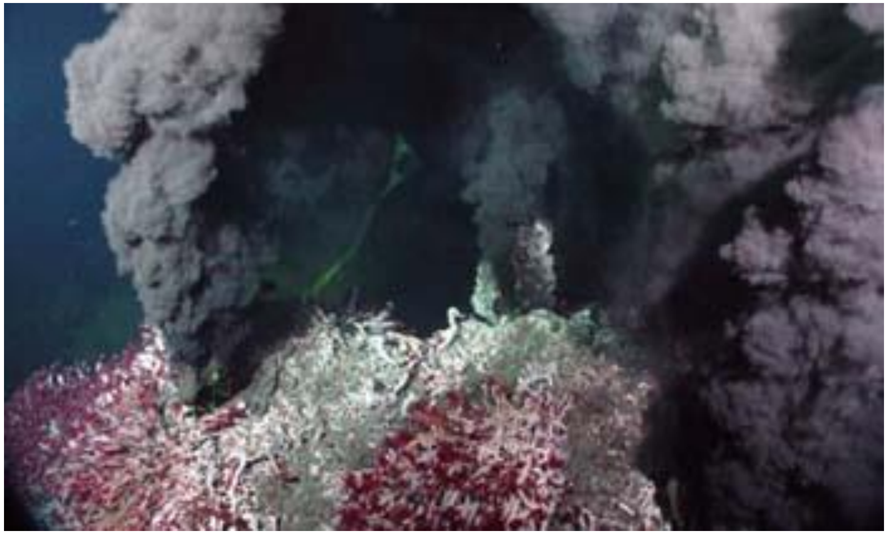
1793 — **Alexander von Humboldt examines fungi in Fichtel Mountains gold mines.**

1911 — **Galle grows microbes from coal mine samples, in search of methanogens.**

1926 — **Bastin grows sulfate reducing microbes from Illinois & California oil field water.**

Reports dismissed by concerns of contamination by surface microbes.

1977: Discovery of deep sea hydrothermal vents (a.k.a Black Smokers)



NOAA

Base of food chain:

(Surface) Photosynthethic organisms use sunlight to produce organic molecules.

(Black Smokers) Microbes consume H_2S, CO_2, H_2O, … to produce organic molecules.

No solar dependence!

How About in Deep Rock?

Need broad, sustained, systematic studies.
$$$$$

Nuclear Waste to the Rescue!!

1986 Frank Wobber's DOE Subsurface Science Program (SSP) funded <u>systematic tool development and studies</u> of deep life.

Other Deep Lab programs start in Belgium, Canada, Sweden….

Challenge: Do microbiota live in the deep crust?

- Don't assume. Sample & analyze.
- But to sample, either tunnel or drill – neither is sterile. Coring tools.
- Tracers. Perfluocarbon to the rescue!
- How to find a few cells in a kilogram of rock? Especially if they won't grow in culture.
- Polymerase Chain Reaction (PCR) & genetic advances to the rescue!

Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 6045–6049, July 1992
Microbiology

The deep, hot biosphere

(geochemistry/planetology)

THOMAS GOLD

Cornell University, Ithaca, NY 14853

Contributed by Thomas Gold, March 13, 1992

ABSTRACT There are strong indications that microbial life is widespread at depth in the crust of the Earth, just as such life has been identified in numerous ocean vents. This life is not dependent on solar energy and photosynthesis for its primary energy supply, and it is essentially independent of the surface circumstances. Its energy supply comes from chemical sources, due to fluids that migrate upward from deeper levels in the Earth. In mass and volume it may be comparable with all surface life.

be common as solitary objects in space, as well as in other solar-type systems.

PNAS | July 3, 2017 | vol. 114 | no. 27 | 6895–6903

The deep, hot biosphere: Twenty-five years of retrospection

Daniel R. Colman[a], Saroj Poudel[a], Blake W. Stamps[b], Eric S. Boyd[a,c,1], and John R. Spear[b,c,1]

Edited by David M. Karl, University of Hawaii, Honolulu, HI, and approved May 16, 2017 (received for review January 25, 2017)

Twenty-five years ago this month, Thomas Gold published a seminal manuscript suggesting the presence of a "deep, hot biosphere" in the Earth's crust. Since this publication, a considerable amount of attention has been given to the study of deep biospheres, their role in geochemical cycles, and their potential to inform on the origin of life and its potential outside of Earth. Overwhelming evidence now supports the presence of a deep biosphere ubiquitously distributed on Earth in both terrestrial and marine settings. Furthermore, it has become apparent that much of this life is dependent on lithogenically sourced high-energy compounds to sustain productivity. A vast diversity of uncultivated microorganisms has been detected in subsurface environments, and we show that H_2, CH_4, and CO feature prominently in many of their predicted metabolisms. Despite 25 years of intense study, key questions remain on life in the deep subsurface, including whether it is endemic and the extent of its involvement in the anaerobic formation and degradation of hydrocarbons. Emergent data from cultivation and next-generation sequencing approaches continue to provide promising new hints to answer these questions. As Gold suggested, and as has become increasingly evident, to better understand the subsurface is critical to further understanding the Earth, life, the evolution of life, and the potential for life elsewhere. To this end, we suggest the need to develop a robust network of interdisciplinary scientists and accessible field sites for long-term monitoring of the Earth's subsurface in the form of a deep subsurface microbiome initiative.

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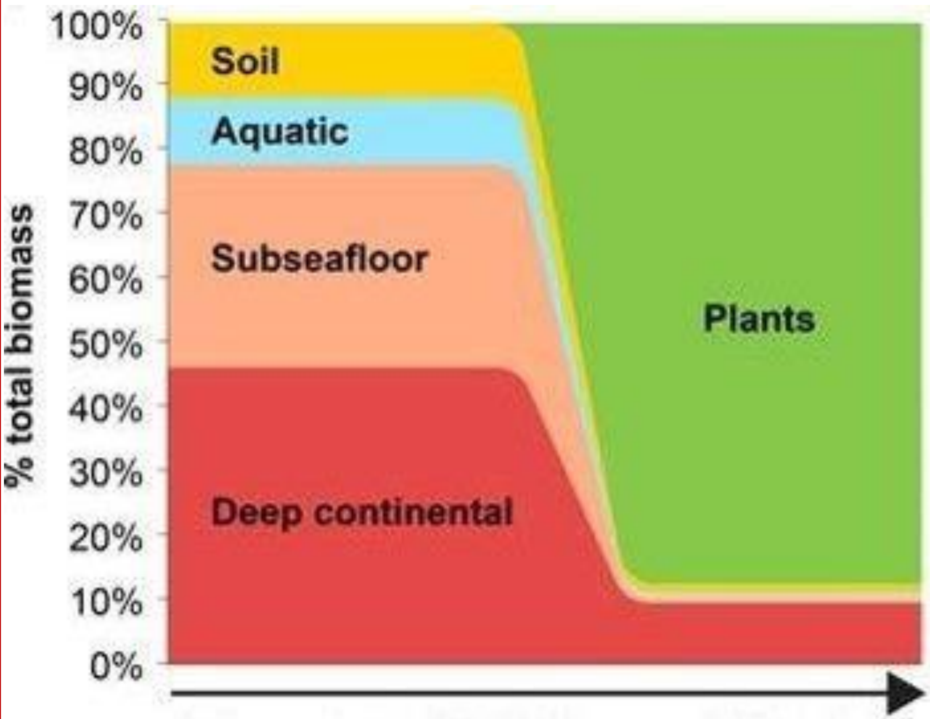
What's the Energy Source?

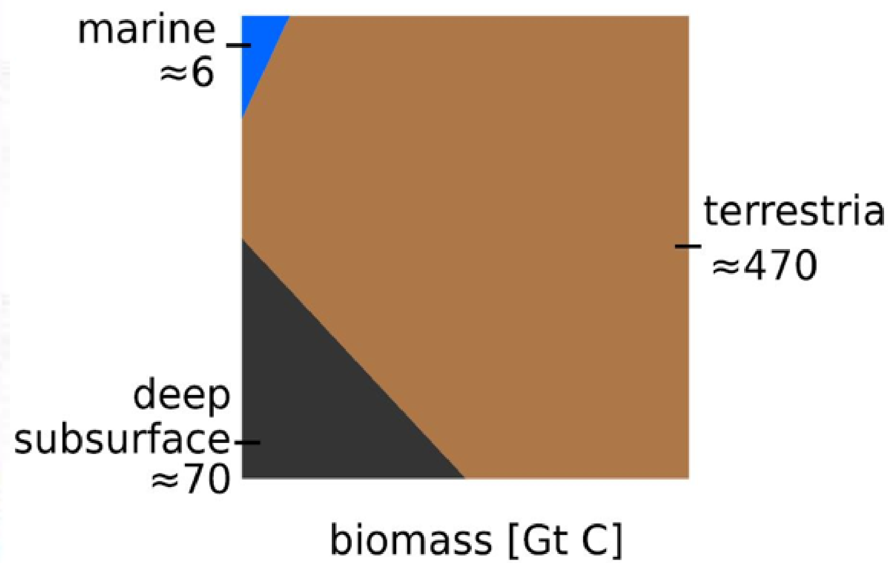
- **Earth is not yet in full thermodynamic equilibrium. There are reactive minerals, primordial heat, and energy influx of radioactive decay.**
- **1992: An anaerobic, thermophilic bacterium, dubbed *Bacillus infernus* isolated from a deep coring site.**
- **1993, International Symposium on Subsurface Microbiology (Bath, England): Radiolysis of water => H_2, which directly feeds *Bacillus infernus*.**

How much biomass is down there?

Estimates: from a few percent to a majority of Earth's biomass!



McMahon & Parnell, *JGS*, **175** (2018) 716.



Bar-On et al., *PNAS* **115** (2018) 6508

How much biomass is down there?

Table 1. Summary of estimated total biomass for abundant taxonomic groups

Taxon	Mass (Gt C)	Uncertainty (-fold)
Plants	450	1.2
Bacteria	70	10
Fungi	12	3
Archaea	7	13
Protists	4	4
Animals	2	5
Viruses	0.2	20
Total	550	1.7

Bar-On et al., *PNAS* **115** (2018) 6508

Grand Challenge Questions

NOTE

- Answers will be years in the making.
- Data and papers coming at a rapid rate.
- Snapshot in time of progress.

Grand Challenge Questions

- **Did life start in Hell, e.g., deep down at Hi-P, Hi-T?**

Life Itself May Have Originated Under Pressure

nature microbiology | ARTICLES

PUBLISHED: 25 JULY 2016 | ARTICLE NUMBER: 16116 | DOI: 10.1038/NMICROBIOL.2016.116

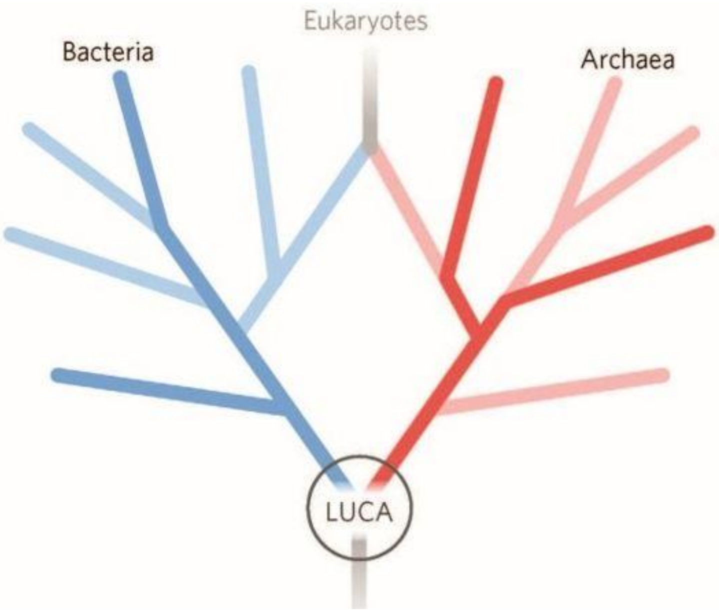
The physiology and habitat of the last universal common ancestor

Madeline C. Weiss†, Filipa L. Sousa†, Natalia Mrnjavac, Sinje Neukirchen, Mayo Roettger, Shijulal Nelson-Sathi and William F. Martin*

REVIEW

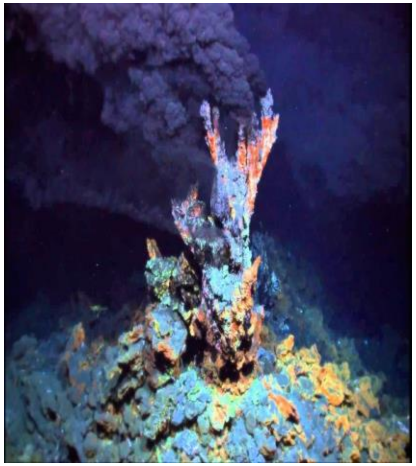
PLOS | GENETICS 14 (2018) e1007518

Madeline C. Weiss[1], Martina Preiner[1], Joana C. Xavier[1], Verena Zimorski[1], William F. Martin[1,2]*



Conclusions

gave rise to the first biochemical pathways that in turn gave rise to the first cells? Genes that trace to LUCA [78], ancient biochemical pathways [103], and aqueous reactions of CO_2 with iron and water [98,110] all seem to converge on similar sets of simple, exergonic chemical reactions as those that occur spontaneously at hydrothermal vents [148]. From the standpoint of genes, physiology, laboratory chemistry, and geochemistry, it is beginning to look like LUCA was rooted in rocks.



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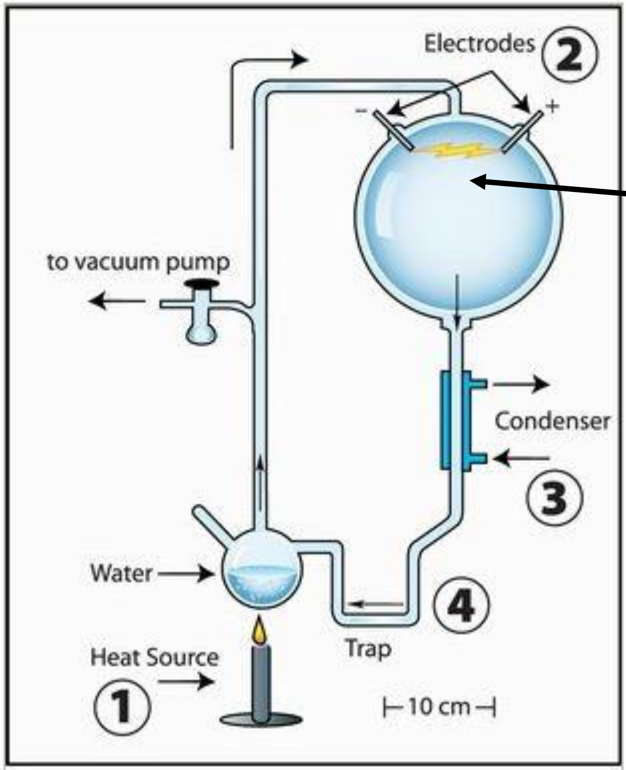

Credit: Ned Shaw, Indiana University

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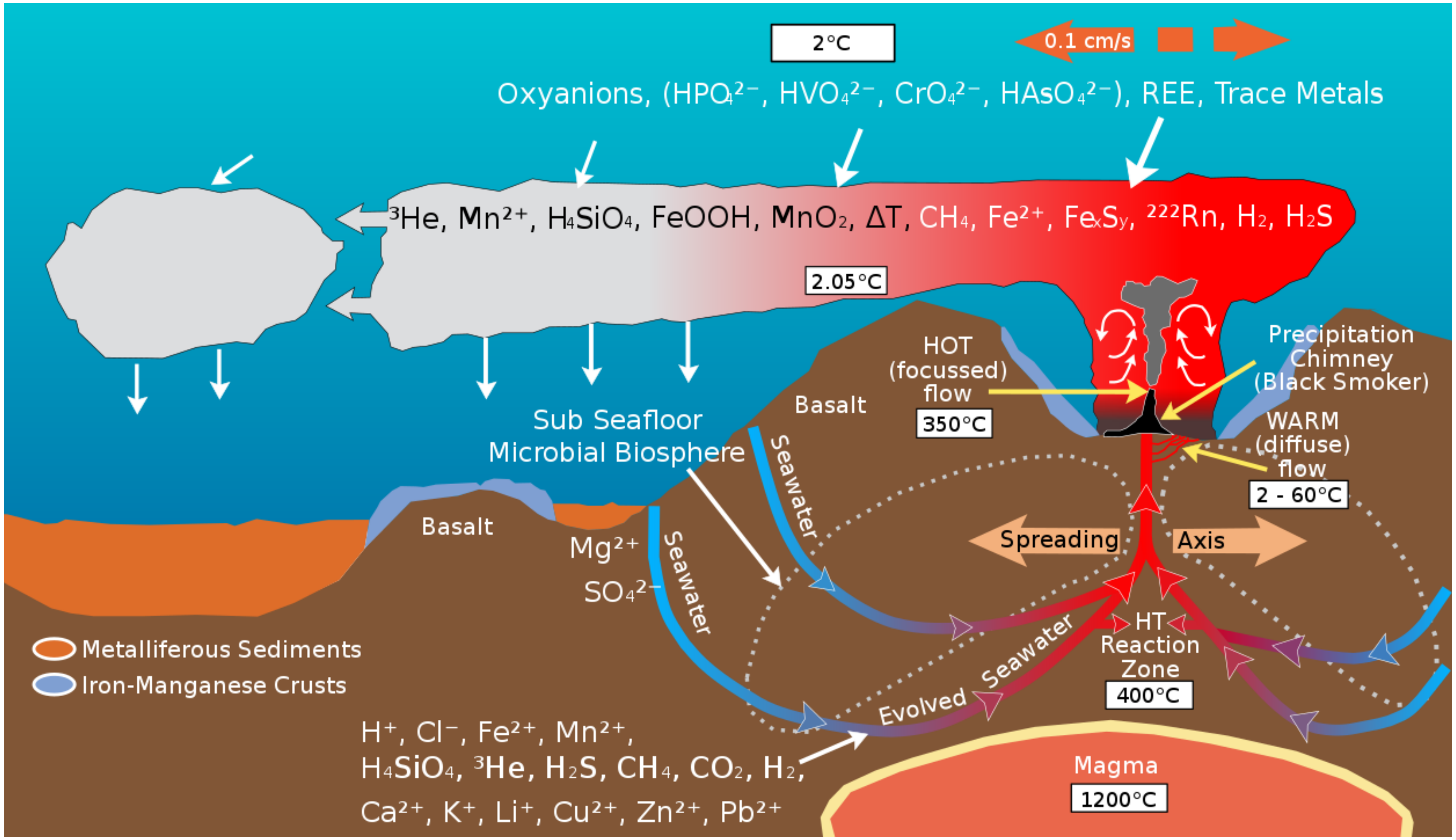
Need Energy Gradients & Host Catalysts



Wikipedia: "Hydrothermal Vents"

See:

- Branscomb, Biancalani, Goldenfeld & Russell, *Physics Reports*, **677** (2017) 1
- Branscomb & Russell, *BioEssays*, **40** (2018) 1700182
- Duval et al., *Interface Focus* **9** (2019) 20190063

Hydrothermal Vent Fossils?

Nature, **453** (2017) 60

Evidence for early life in Earth's oldest hydrothermal vent precipitates

Matthew S. Dodd[1,2], Dominic Papineau[1,2], Tor Grenne[3], John F. Slack[4], Martin Rittner[2], Franco Pirajno[5], Jonathan O'Neil[6] & Crispin T. S. Little[7]

Although it is not known when or where life on Earth began, some of the earliest habitable environments may have been submarine-hydrothermal vents. Here we describe putative fossilized microorganisms that are at least 3,770 million and possibly 4,280 million years old in ferruginous sedimentary rocks, interpreted as seafloor-hydrothermal vent-related precipitates, from the Nuvvuagittuq belt in Quebec, Canada. These structures occur as micrometre-scale haematite tubes and filaments with morphologies and mineral assemblages similar to those of filamentous microorganisms from modern hydrothermal vent precipitates and analogous microfossils in younger rocks. The Nuvvuagittuq rocks contain isotopically light carbon in carbonate and carbonaceous material, which occurs as graphitic inclusions in diagenetic carbonate rosettes, apatite blades intergrown among carbonate rosettes and magnetite–haematite granules, and is associated with carbonate in direct contact with the putative microfossils. Collectively, these observations are consistent with an oxidized biomass and provide evidence for biological activity in submarine-hydrothermal environments more than 3,770 million years ago.

Hydrothermal Vent Fossils?

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Mat
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Grand Challenge Questions

- Did life start in Hell, e.g., deep down at Hi-P, Hi-T?
- **When did life start? What does it take to sterilize the Earth?**

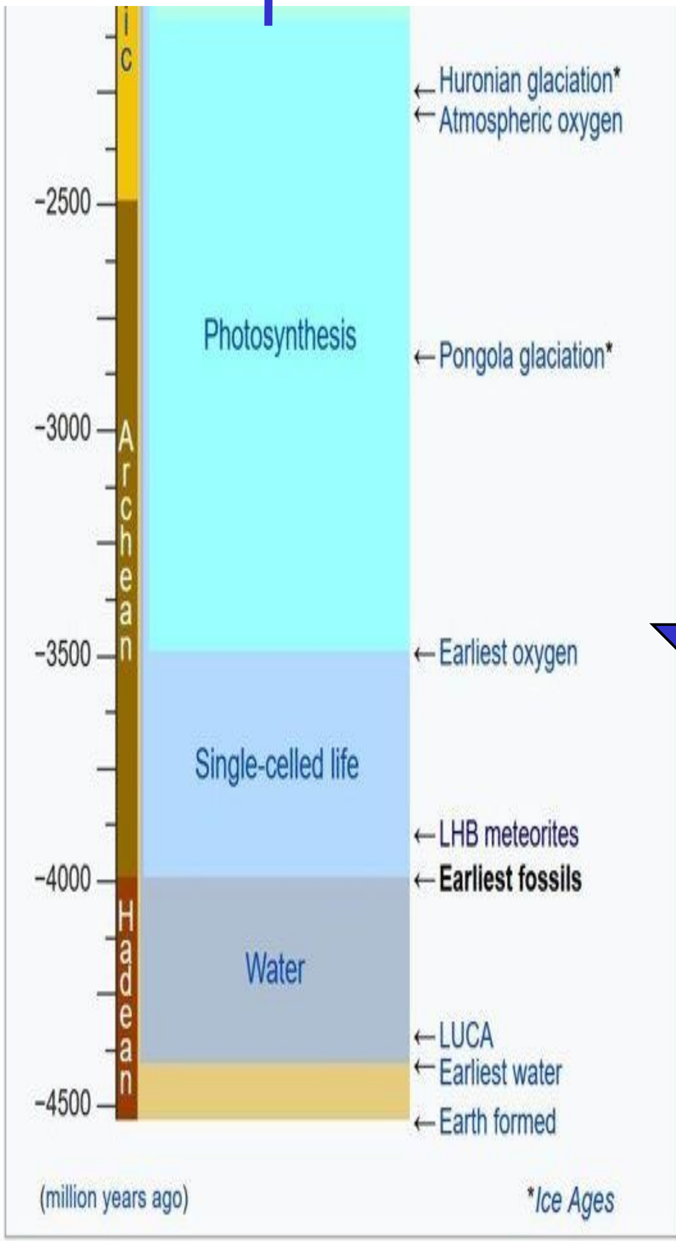
Earth's Chronology

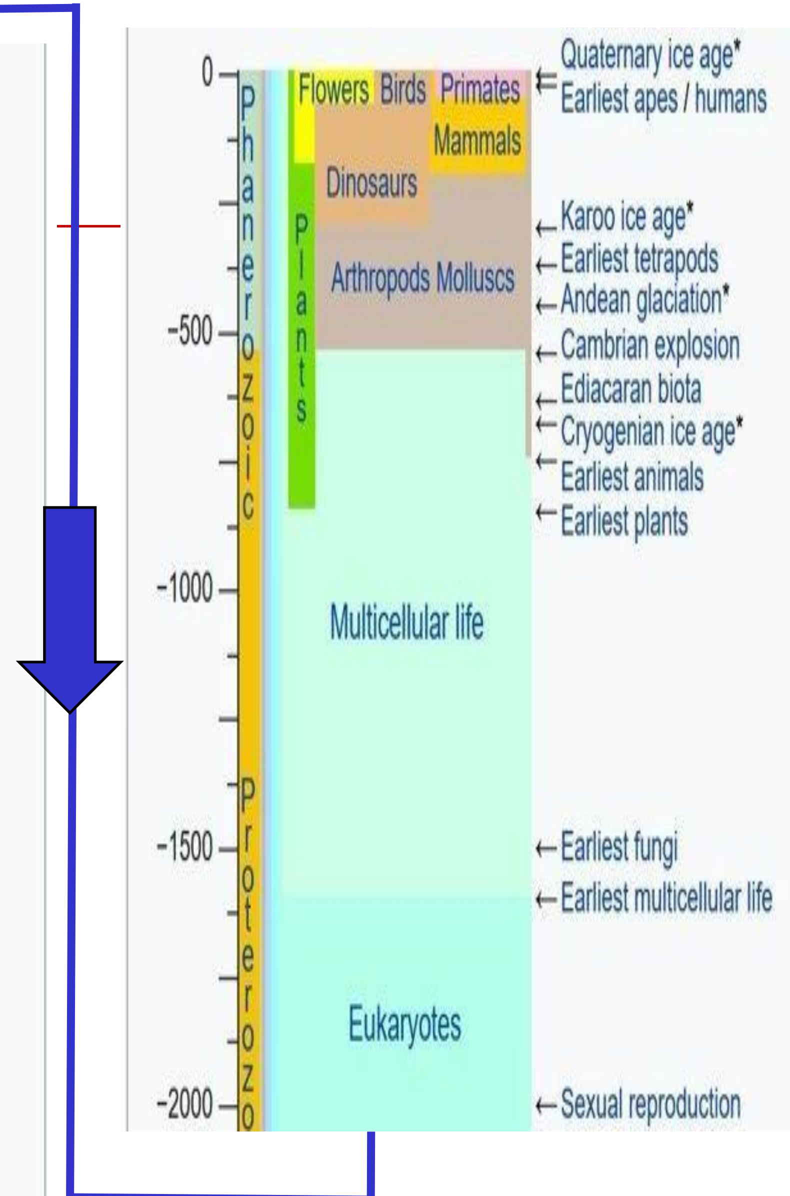
4.54 Ga: Solar system & Earth form. Impact forms moon soon after.

~4.4 Ga: Earth covered by oceans.

< 4 Ga: DNA based life on Earth.

4.1 – 3.9 Ga Late heavy bombardment.





Wikipedia: History of Earth

Late Heavy Bombardment
(~ 4.1 to 3.9 Ga)

Photo by Gregory H. Revera via
http://en.wikiperia.org/wiki/Creative_Commons

LHB impacts on Earth

- >22,000 impact craters with diameters >20 km
- ~40 impact basins with diameters about 1,000 km
- several impact basins with diameters about 5,000 km

Sterilizing the Earth

Vol 459 | 21 May 2009 | doi:10.1038/nature08015 nature

Key: hi-P_Bio

LETTERS

Microbial habitability of the Hadean Earth during the late heavy bombardment

Oleg Abramov[1] & Stephen J. Mojzsis[1]

Lunar rocks[1,2] and impact melts[3], lunar[4] and asteroidal meteorites[5], and an ancient martian meteorite[6] record thermal metamorphic events with ages that group around and/or do not exceed 3.9 Gyr. That such a diverse suite of solar system materials share this feature is interpreted to be the result of a post-primary-accretion cataclysmic spike in the number of impacts commonly referred to as the late heavy bombardment (LHB)[1–7]. Despite its obvious significance to the preservation of crust and the survivability of an emergent biosphere, the thermal effects of this bombardment on the young Earth remain poorly constrained. Here we report numerical models constructed to probe the degree of thermal metamorphism in the crust in the effort to recreate the effect of the LHB on the Earth as a whole; outputs were used to assess habitable volumes of crust for a possible near-surface and subsurface primordial microbial biosphere. Our analysis shows that there is no plausible situation in which the habitable zone was fully sterilized on Earth, at least since the termination of primary accretion of the planets and the postulated impact origin of the Moon. Our results explain the root location of hyperthermophilic bacteria in the phylogenetic tree for survival of incipient life[17] in one or more 'impact frustrations', and

disrupted the crust to such a degree that no Earth rocks survive from before ~3.8 Gyr ago[18]. However, Earth rocks that coincide with or pre-date the LHB are known, and date from as early as 4.03 Gyr ago[19]. Furthermore, geochemical evidence from terrestrial zircons older than 4.0 Gyr points to there having been liquid water, crustal recycling, granitoid crust and low-temperature plate boundary processes throughout the Hadean eon (4.38–3.85 Gyr ago)[20,21]. On the basis of this new perspective of the very early Earth, it makes sense to address whether a biosphere that may have arisen in the Hadean could have survived through the LHB.

To explore the thermal state and habitability of the early Earth during the LHB, we constructed thermal models that incorporate (1) new studies of impact cratering records of the Moon and the terrestrial planets, and the size distributions of asteroid populations[22]; (2) data from a new class of early-Solar-System dynamical models that successfully reproduce impact rates during the bombardment as defined by the lunar and meteoritic record[23]; and (3) powerful numerical methods that explore the thermal response of the lithosphere to

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- When did life start? What does it take to sterilize the Earth?
- **How long is a microorganism viable? How long can a cell go before it reproduces?**

Microbial Life is Incredibly Robust

PNAS

Methyl-compound use and slow growth characterize microbial life in 2-km-deep subseafloor coal and shale beds

E9206–E9215 | PNAS | Published online October 3, 2017

Elizabeth Trembath-Reichert[a,1], Yuki Morono[b,c], Akira Ijiri[b,c], Tatsuhiko Hoshino[b,c], Katherine S. Dawson[a], Fumio Inagaki[b,c,d], and Victoria J. Orphan[a,1]

[a]Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125; [b]Geomicrobiology Group, Kochi Institute for Core Sample Research, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Monobe B200, Nankoku, Kochi 783-8502, Japan; [c]Geobiotechnology Group, Research and Development Center for Submarine Resources, JAMSTEC, Monobe B200, Nankoku, Kochi 783-8502, Japan; and [d]Research and Development Center for Ocean Drilling Science, JAMSTEC, Yokohama, Kanagawa 236-0001, Japan

- **20M year old coal, 2 km below sea floor**
- **Contained living microbes**
- **16S rRNA phylogenic marker genes were from forest, not marine cells**
- **Living slowly – Incubation time estimates were a few months to hundreds of years**

…Really, Really Robust!

letters to nature

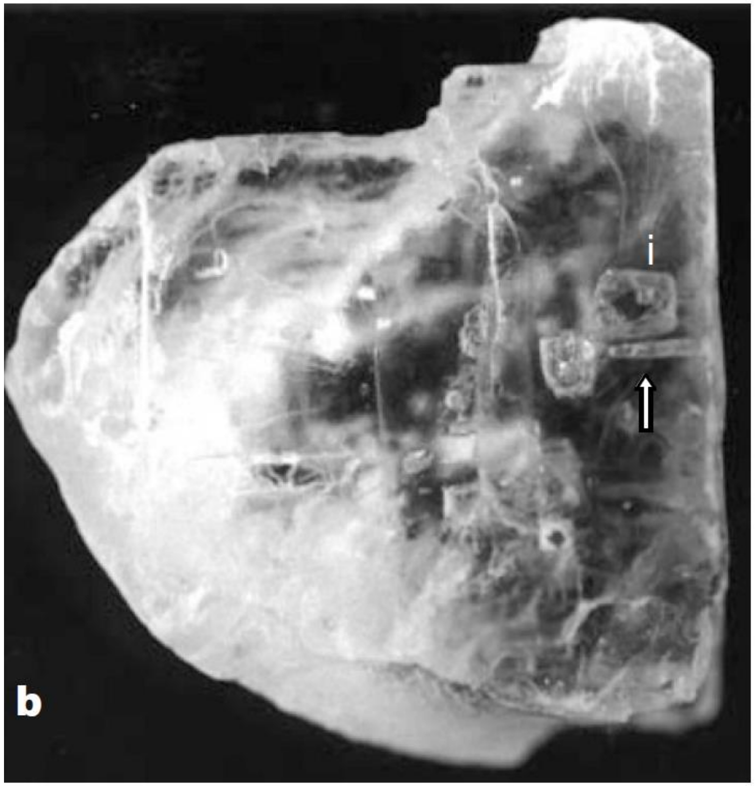
NATURE | VOL 407 | 19 OCTOBER 2000 | www.nature.com

Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal

Russell H. Vreeland*, William D. Rosenzweig* & Dennis W. Powers†

* *Department of Biology, West Chester University, West Chester, Pennsylvania 19383, USA*
† *Consulting Geologist, Box 87, Anthony, Texas 79821, USA*

Bacteria have been found associated with a variety of ancient samples[1], however few studies are generally accepted due to questions about sample quality and contamination. When Cano and Borucki[2] isolated a strain of *Bacillus sphaericus* from an extinct bee trapped in 25–30 million-year-old amber, careful sample selection and stringent sterilization techniques were the keys to acceptance. Here we report the isolation and growth of a



…Really, Really Robust!

sample selection and stringent sterilization techniques were the keys to acceptance. Here we report the isolation and growth of a previously unrecognized spore-forming bacterium (*Bacillus* species, designated 2-9-3) from a brine inclusion within a 250 million-year-old salt crystal from the Permian Salado Formation. Complete gene sequences of the 16S ribosomal DNA show that the

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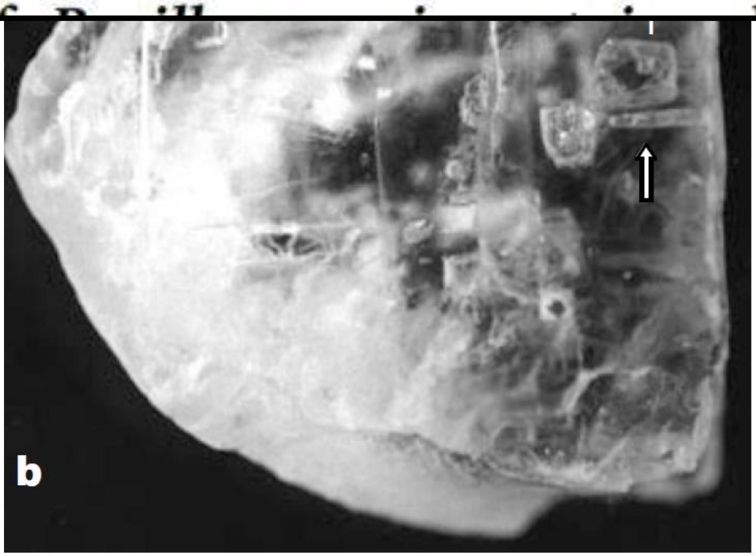

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Dombrowski, H., *Annals of the New York Academy of Sciences*, 108 (1963) 453-460.

BACTERIA FROM PALEOZOIC SALT DEPOSITS

Heinz Dombrowski
Justus-Liebig University, Giessen, Germany

Stimulated by the bacteriological findings in the mineral springs of Bad Nauheim, which carry salts from Permian deposits, I investigated from a bacteriological point of view the Zechstein salts, obtained by means of mining and drilling. Müller and Schwartz (1953), Rippel (1945), and Strong (1956) only achieved the isolation of dead bacteria from Zechstein salts. Reiser and Tasch (1960) reported the living isolation of a diplococcus from Permian salts. We now succeeded in isolating living bacteria. Yet, this achievement seemed rather improbable; for if we had actually extracted living bacteria from Zechstein salts, then we have to assume that we found creatures of the highest individual age ever registered.

…Really, Really, Really, Really Robust!

GEOLOGY | THE GEOLOGICAL SOCIETY OF AMERICA®

https://doi.org/10.1130/G49957.1

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830-million-year-old microorganisms in primary fluid inclusions in halite

Sara I. Schreder-Gomes*, Kathleen C. Benison and Jeremiah A. Bernau†
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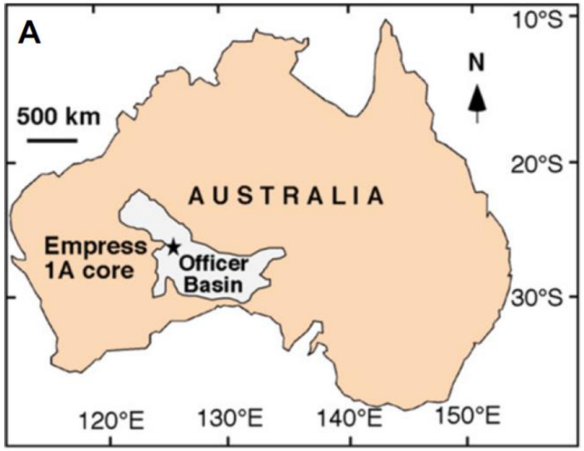

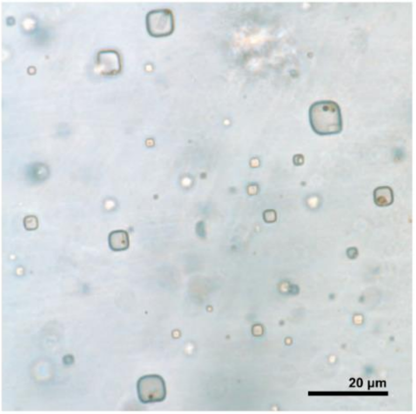

Figure 4. Distribution of microorganisms in primary fluid inclusions in Browne Formation halite (central Australia) from the Empress 1A core, at 1520.1 m depth.

…Really, Really, Really, Really Robust!

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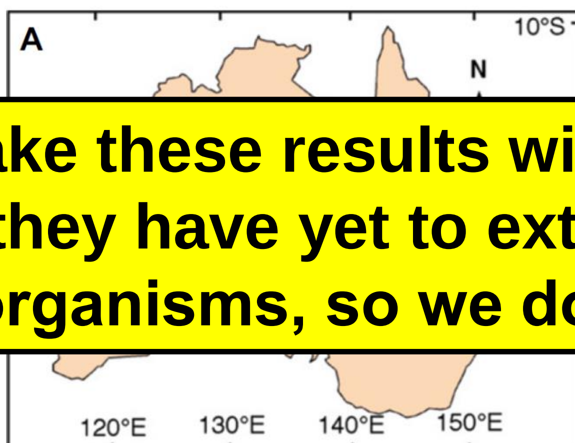

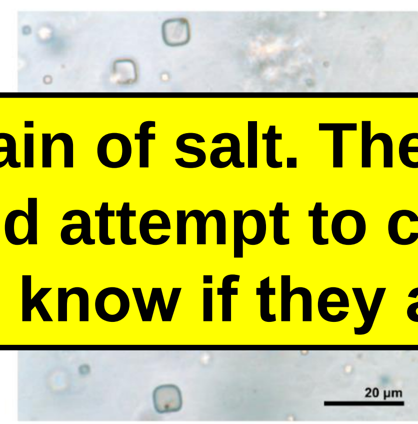

But take these results with a grain of salt. The authors admit they have yet to extract and attempt to culture the microorganisms, so we don't yet know if they are viable.

Figure 4. Distribution of microorganisms in primary fluid inclusions in Browne Formation halite (central Australia) from the Empress 1A core, at 1520.1 m depth.

Grand Challenge Questions

- Did life start in Hell, deep down at Hi-P, Hi-T?
- When did life start?
- How long is a microorganism viable? How long before it reproduces?
- **What are pressure and temperature limits of life?**

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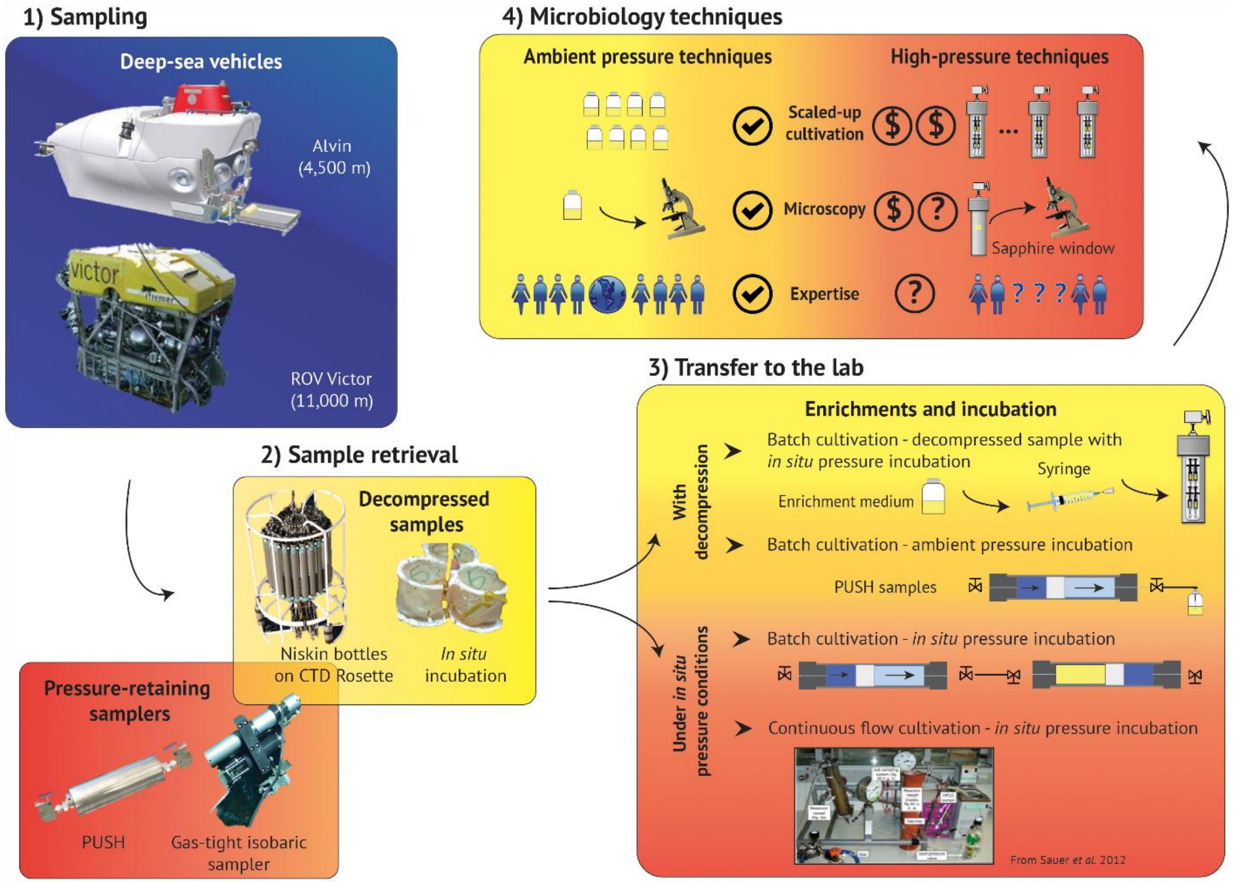
If it did, how would we know it?

Grand Challenge Questions

- **How do extremophiles differ from surface organisms?**



A. Cario, G.C. Oliver & Karyn L. Rogers, *Exploring the Deep Marine Biosphere: Challenges, Innovations, and Opportunities*, **Frontiers in Earth Science**, 7 (2019) 225.

Grand Challenge Questions

- How do extremophiles differ from surface organisms?

- What are the biophysics of biomolecules under Hi-P and Hi-T?

Work has been done on Hi-T; relatively little on Hi-P molecular structure.

Determination of changes in biomolecule structure with pressure is the subject of two other lectures. Here is a teaser….

You have had too much to drink and need to drive home.

Question: How can you quickly get sober?

Answer: Quickly pressurize your car to a few hundred atmospheres.

(1 atmosphere = 1 bar = 10^5 Pa = 10^5 N/m^2)

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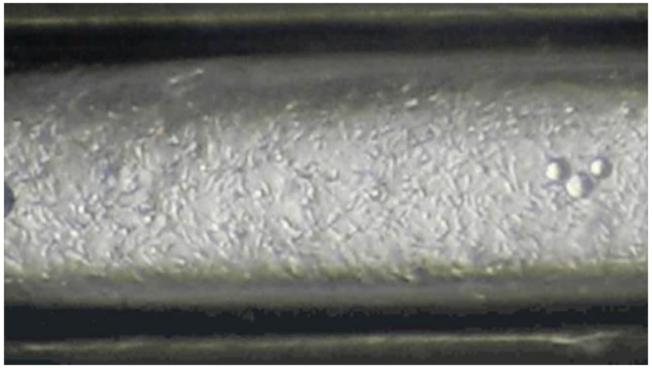

1 atm

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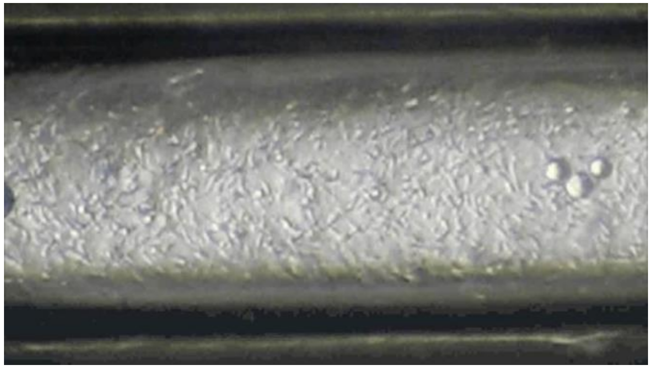

1 atm

1500 atm

What's Going on Here?

- **Pressure reversal of anaesthesia has been known for most of a century. Still not understood.**
- **Understanding pressure effects at the <u>whole organism</u> level is too complicated.**
- **Better: Start by understanding pressure effects on <u>biomolecular structure</u>.**

Pressure Affects Biomacromolecules in Numerous Ways

- **Pressure unfolding of proteins (Bridgman – 1914)**
- **Multimer association/disassociation**
- **Changes in ligand & small molecule binding**
- **Altered membrane ion transduction**
- **Changes in transcription of nucleic acids**
- **Large shifts in chemical kinetic constants**
- **Changes in conformational states**
- **Greatly decreased viral infectivity**

…. i.e., much of the machinery of life.

These are the topics of other lectures.

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I've been fortunate to enjoy working with many fantastic students, post-docs and colleagues. In the pressure area, I thank:

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END OF LECTURE

Life Itself May Have Originated Under Pressure

nature microbiology

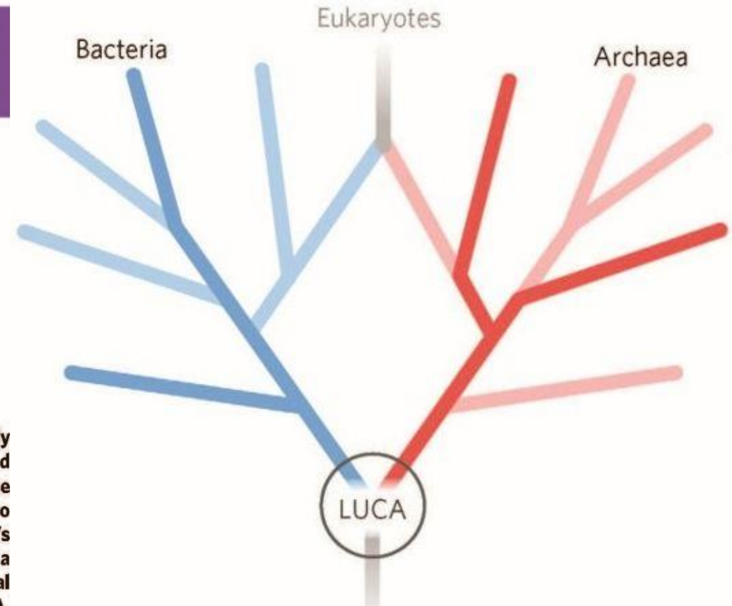
ARTICLES

PUBLISHED: 25 JULY 2016 | ARTICLE NUMBER: 16116 | DOI: 10.1038/NMICROBIOL.2016.116

The physiology and habitat of the last universal common ancestor

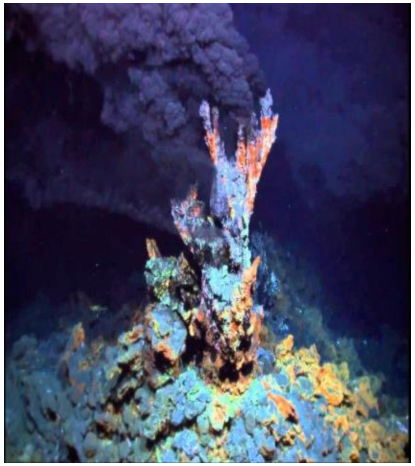
Madeline C. Weiss†, Filipa L. Sousa†, Natalia Mrnjavac, Sinje Neukirchen, Mayo Roettger, Shijulal Nelson-Sathi and William F. Martin*

The concept of a last universal common ancestor of all cells (LUCA, or the progenote) is central to the study of early evolution and life's origin, yet information about how and where LUCA lived is lacking. We investigated all clusters and phylogenetic trees for 6.1 million protein coding genes from sequenced prokaryotic genomes in order to reconstruct the microbial ecology of LUCA. Among 286,514 protein clusters, we identified 355 protein families (~0.1%) that trace to LUCA by phylogenetic criteria. Because these proteins are not universally distributed, they can shed light on LUCA's physiology. Their functions, properties and prosthetic groups depict LUCA as anaerobic, CO_2-fixing, H_2-dependent with a Wood–Ljungdahl pathway, N_2-fixing and thermophilic. LUCA's biochemistry was replete with FeS clusters and radical reaction mechanisms. Its cofactors reveal dependence upon transition metals, flavins, *S*-adenosyl methionine, coenzyme A, ferredoxin, molybdopterin, corrins and selenium. Its genetic code required nucleoside modifications and *S*-adenosyl methionine-dependent methylations. The 355 phylogenies identify clostridia and methanogens, whose modern lifestyles resemble that of LUCA, as basal among their respective domains. LUCA inhabited a geochemically active environment rich in H_2, CO_2 and iron. The data support the theory of an autotrophic origin of life involving the Wood–Ljungdahl pathway in a hydrothermal setting.



Discussion

Our findings clearly support the views that FeS and transition metals are relics of ancient metabolism[23,24], that life arose at hydrothermal vents[12,13], that spontaneous chemistry in the Earth's crust driven by



Oregon State University / CC BY-SA 2.0.

Life Itself May Have Originated Under Pressure

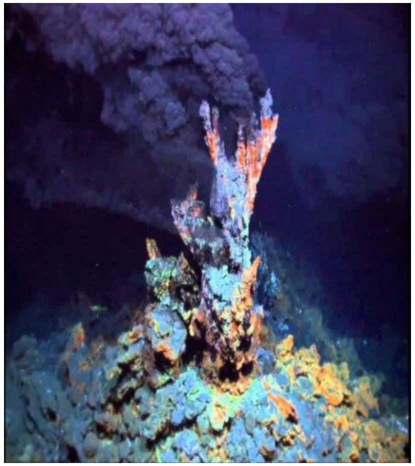
Conclusions

gave rise to the first biochemical pathways that in turn gave rise to the first cells? Genes that trace to LUCA [78], ancient biochemical pathways [103], and aqueous reactions of CO_2 with iron and water [98,110] all seem to converge on similar sets of simple, exergonic chemical reactions as those that occur spontaneously at hydrothermal vents [148]. From the standpoint of genes, physiology, laboratory chemistry, and geochemistry, it is beginning to look like LUCA was rooted in rocks.

REVIEW

PLOS | GENETICS **14** (2018) e1007518

Madeline C. Weiss[1], Martina Preiner[1], Joana C. Xavier[1], Verena Zimorski[1], William F. Martin[1,2]*



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