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From self-assembly of life to present-day bacteria: a possible role for nanocells

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Abstract

A proposed sequence of major events for the self-assembly of life on Earth is examined. This sequence starts with a construction kit of elements and simple compounds from which a primitive membrane and then a nanocell with a minimal genome is self-assembled. The genome and cell increase in size and complexity and become capable of cell division, similar to present-day bacteria. Another factor to understanding this self-assembly of life is identifying the energy source(s) the first self-assembling nanocells were capable of using. This will also be examined from an evolutionary perspective with hydrogen as the postulated universal energy source [Morita, R. (2000) Microb. Ecol. 38, 307–320].

Keywords: Assembly; Bacterium; Earth; Energy; Evolution; Hydrogen; Life; Nanobacterium; Nanocell; Origin; Sequence

Contents

1. Introduction

Life probably started after cellular evolution reached a level of complexity that allowed the subsequent diversification of life [1]. Also, the order of major events that allowed the first cell capable of growth and division to assemble is central to the understanding of the origin of life on Earth. If the formation of a primitive membrane, vesicle or microsphere in a hydrophobic medium (HM) [2,3] was one of the first self-assembly events in the origin of life, then a central requirement for a cell, an inside and outside, was achieved. This type of structure would provide physical containment for subsequent cellular macromolecules. For primitive pre-biotic cells to further self-assemble, they would need to remain stable while other biomolecules assembled at the same physical location or were transported to the cells, or the assembling cells were

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transported to a location where other necessary molecules were available. If life originated on the Earth, then some or all of these possibilities must have occurred and scientists are confronted with the challenges of understanding these processes with minimal to no data available. It is highly probable the formation of a primitive membrane or microsphere [4] occurred without assistance from enzymes. Once this event had occurred, the remaining components of the first cells, possibly nanocells, self-assembled in a more biochemically stable environment protected from the external environment. It is also possible that nanocells were not uniform in size, but varied, and acted as a cooperative biomass or biofilm on a mineral surface at an air–liquid interface.

Nanobacteria are typically 0.2–0.5 μm in diameter but can also pass through 0.1-μm filters because smaller forms of 0.05–0.2 μm have also been observed with transmission electron microscopy (TEM) [5], although it is not certain whether these structures are living cells. The transition from a primitive nanocell to nanocells with a minimal genome followed by molecular optimization [6,7] may have been the major route life used to arrive at present-day bacterial cells.

Knowledge of the self-assembly of the first living cell is central to understanding live on Earth and elsewhere [7–9]. Self-assembly is defined as the first membrane-bound structure capable of self-replication, mutation and then replication in a mutated state [1]. The cell as the basic unit and a genome capable of relative constancy with some changes over time was the mechanism life used to further evolve and diversify. The knowledge gaps in this process are immense. One way of viewing our limited knowledge is that we do not know what we do not know. A paucity of knowledge also exists on the molecular organizing mechanisms and the scales (from molecular, nanocell, micron cellular and globally from chemosphere to living biosphere) that were operating during self-assembly of the first cell or the last universal common ancestor [1], and then dispersal, survival and colonization of the cells over the Earth to commence the formation of a complex biosphere.

Evolution would be virtually impossible without the cell as the basic unit of life [10]. What structure other than a cell could self-assemble, contain a genome and be capable of division? The cell has an inside and outside necessary to separate it from its often harsh or extreme physical–chemical external environment. Cells also must generate, store and use energy. This eventually requires complexity. The

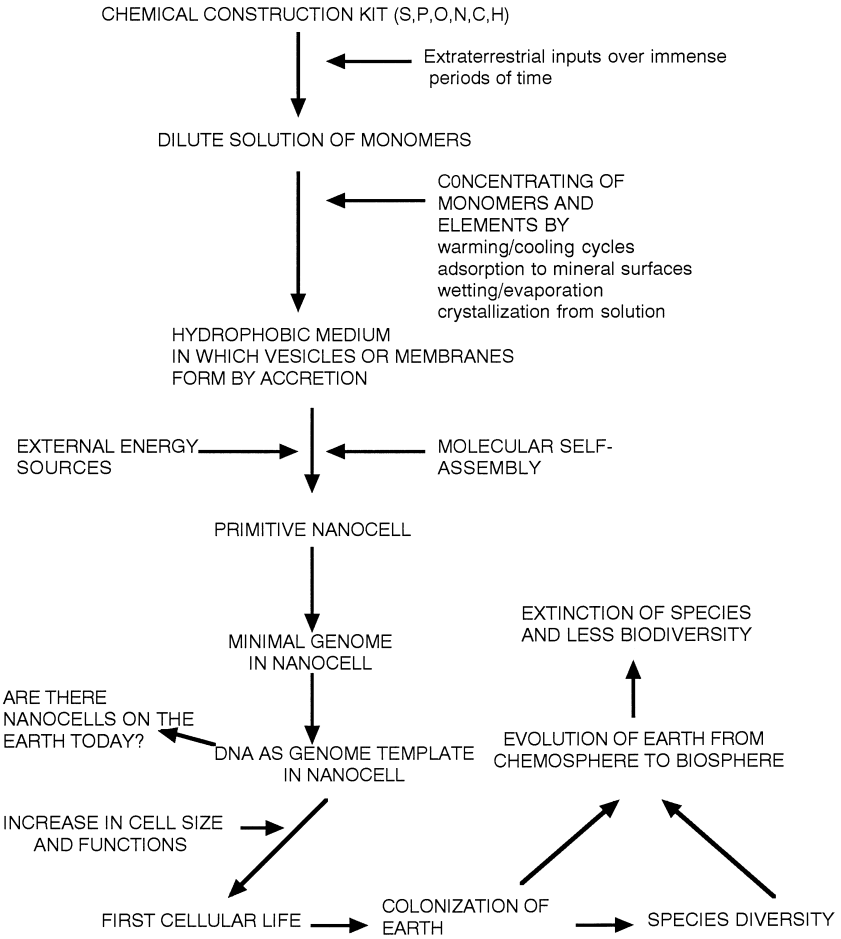

Fig. 1. Proposed sequence of major events in the origin of a cell capable of growth, division and diversification.

abundance of the first 31 elements in the Periodic Table on Earth and in the known universe is highly correlated: Peusner [11] estimated the self-assembly of a primitive cell occurring as a spontaneous act, where the molecules and elements were present at a specific location on the Earth in a suitable sequence, as 10^{-254}. Conversely, even a highly improbable event can occur. This only adds to the paucity of information and the confusion we struggle with when researching the origin of life. According to Peusner [11] this suggests the origin of life and evolution occurred in an ordered manner from the simple to the more complex.

The self-assembly events that led to the first minimal cell and genome capable of growth and division are highly debated. Fig. 1 is a proposed sequence of major events that may have occurred initially at a molecular level and then progressed to a nanocell level and finally to the bacterial cell dimensions (μm) that we know today. In this review we will examine the major self-assembly events for cells as outlined in Fig. 1. We will also discuss the possibility that nanobacteria, which are small spherical and ovoid structures discovered in rocks and minerals, may be the fossil evidence of the earliest life forms on Earth and outer space. Fig. 1 also indicates a role for extraterrestrial inputs which may have included living spores. If spores arrived on the Earth from an extraterrestrial source, then many of the mechanisms proposed in the remainder of this article would not have occurred on the Earth. Life could have originated on the Earth, while another possibility is that life arrived on the Earth as spores from an extraterrestrial source. Spores could have provided the protection needed from ultraviolet light and radiation for 4.5–45 million years in outer space, allowing sufficient time to contact the Earth [12]. The origin of spores and their dispersal into space could have been a collision between some planet on which life existed and a meteorite [13]. This should be considered as possible as the origin of life on the Earth. For the remainder of this article, we will deal with the possible origin of life on the Earth.

If the origin of life did occur on the Earth, the location of the self-assembly of life may never be known. For example, it has been suggested that ocean vents (temperatures around 350°C) that release hot water, CO_2, CO, CH_4, H_2S and NH_3 and metal sulfides may have been suitable for sites for the origin of life on Earth [14]. This type of extreme ancient environment may be a possible location for the origin of life or even dead-end evolution. There is no current means to determine the truth. An extreme environment presents difficulties with respect to the self-assembly of a primitive cell and the origin of catalytic proteins.

Regarding time, it is possible that pre-biotic evolution proceeded at a fast pace. If the age of the Earth is 4.5 billion years and the first 'cell' (visible life forms in ancient rocks) dates back to 3.8 billion years, there was not so much time for evolution: the young Earth must have started cooling and shortly thereafter the first 'cell' appeared.

This review will examine self-assembly, nanobacteria [5,15,16] and hydrogen as a possible universal energy source in the origin and evolution of the first cells. Central to this understanding of early life is the energy source that the cells were capable of using for billions of years of cellular metabolism and the molecular order in which assembly of the cell occurred. A possible universal energy source is hydrogen which has recently been the subject of an excellent review by Morita [17] and also discussed by Trevors [18].

2. Simple compounds and membranes in a HM

2.1. Possible mechanisms to concentrate compounds

The Earth was transformed from a lifeless chemosphere to a complex, living biosphere with immense species, genetic and functional diversity by the origin and evolution of life [18]. Moreover, the elements and simple compounds from which life originated were subject to physical and chemical laws, especially thermodynamic principles. Also, primitive pre-biotic systems would not have been complex with high-fidelity, genetic coding mechanisms for accurate self-replication that is characteristic of cellular life as we understand it today.

Planetary accretion gave rise to certain physical–chemical conditions on the early Earth that were necessary for the assembly of life in a reducing, anoxic environment of hydrogen, ammonia and methane. For life to self-assemble, it required a supply of chemicals (selected elements of the Periodic Table) in concentrations that were not toxic to life but yet sufficient in concentrations for self-assembly of the first cell and then higher organisms. A sufficient supply of chemicals dictated that mechanisms evolved to accumulate or concentrate specific chemicals essential for life [10,19].

Some early possibilities may have been repeated cycles of wetting by dilute solutions and drying at higher temperatures on surfaces of minerals or clays and crystallization of compounds from solutions. Crystallization is the simplest form of self-assembly. Moreover, crystallization provides a way for an ordered structure to self-assemble [10]. Minerals can bind and concentrate elements and compounds necessary for the assembly of the first cell [20]. The ability to bind and concentrate chemicals required for the assembly of cells from dilute aqueous solutions or the interfaces between aqueous solutions and a HM may have been functioning in the origin of life. Also, chemical synthesis leading to the self-assembly of life may have evolved on clay or mineral surfaces [21,22].

The early conditions on the Earth were chemically and

physically harsh and extreme. However, these conditions were suitable for anaerobic bacteria to self-assemble, grow, divide, evolve and diversify. Possibly a starting point for life was self-assembly of a microsphere or membrane in a HM that proceeded to assembly self-replicating reactions for synthesis of the first nanocells. A lipid world scenario as an early step in molecular evolution has been proposed by Lancet et al. [23]. An early step in evolution may have been the ability to make and store energy which required a membrane or microsphere structure for isolating energy rich chemical compounds (e.g. ATP) from the environment. The uptake (both passive and active) of nutrients from the environment and production of ATP are mediated by membranes.

It is possible that a HM of hydrocarbons was a suitable environment for the origin of life, because it would be suitable for polymerization reactions as opposed to hydrolysis reactions in water that would be conducive for decomposition reactions. The physical dimensions of such a HM may have been the same size as bacterial cells (μm dimensions or smaller) or much larger, i.e. they may have covered areas visible to the eye, for instance on the surface of minerals or oceans. Although the origin of life has often been postulated to have occurred in water, it is not unreasonable to consider that life originated in a HM where hydrophobic amino acids concentrated and polymerized [2]. Such a medium may also have been advantageous for the formation of a primitive membrane, vesicles or microspheres which originally gave the first cells their nanocellular morphology. The formation of a primitive membrane or microsphere with an inside and outside must have been a major event en route to the initial assembly of the first nanocells.

2.2. Aerosols

One needs a mechanism for concentrating reactive substances and catalysts to allow polymerization of amino acids and other compounds. Dobson et al. [24] suggested the formation of aerosol particles at the surfactant-covered ocean–atmosphere interface, whereby polar heads are directed inside and hydrophobic tails outside the spheres. During their atmospheric life phase, the core containing an aqueous solution of minerals and small organics can be concentrated and nucleotides and amino acids may undergo a polymerization reaction. These atmospheric aerosols can thus serve as pre-biotic chemical reactors for time periods between 1 day and 1 year (depending on the diameter of the aerosols). In the reducing, pre-biotic atmosphere, OH, NH_2, CH, CH_2, CH_3 and SH radicals were supposedly present, and solar wavelengths down to wavelengths of 174 nm could have induced reactions that would not have normally occurred in the oceanic environment. At their re-entry into the ocean, these spheres, with diameters between 10^{-7} and 10^{-6} m, i.e. in the typical size range of nanobacteria, could have acquired a second layer – now with the hydrophilic end outwards – and persisted in the ocean, sheltered from radiation.

This mechanism allows both for enhanced concentration, crystallization and polymerization processes under harsh atmospheric conditions, and exchange of water, salts, metals and small organic molecules during pelagic conditions, as well as coagulation and merging of microspheres. The model is based on repeated transitions of spheres between chemically reactive conditions (which would be adverse for most extant organisms) and the pelagic milieu which is more propitious to the evolution of living cells. In addition, it also conforms with the cell sizes found for current bacteria and archaea (0.1–10 μm).

2.3. Energy sources

There is additional information that supports the self-assembly of life in a HM. For example, heating a mixture of four amino acids in a mineral oil medium at temperatures from 140 to 360°C yielded small polypeptides [25]. This type of environment and reaction may have been possible on the early Earth. Moreover, no enzymes were required for the peptide formation. This type of thermosynthesis required heat as the mechanism to drive the reaction that produced polymers of simple organic molecules. Heating and drying have also been used to drive polymerization of oligonucleotides [19]. It is not unreasonable to propose that heat or heating/cooling cycles are a major mechanism for driving peptide synthesis in a primordial HM. Other forms of energy such as chemical energy, light, pH gradients for membranes and energy stored in high energy phosphate bonds would not be necessary at this early stage of self-assembly. Muller [26] suggested thermosynthesis of ATP during thermal cycling. Thermosynthesis is a possible candidate as an energy source for the first microorganisms as they function essentially as an open system heat machine.

A sulfur [27] or iron–sulfur chemosphere [28] has also been suggested as an environment suitable for the origin of life. The postulate is that the presence of FeS was used in the self-assembly of life because FeS is needed for metabolic functions such as the acetic acid pathway and assembly of amino acids by reductive amination [28]. It has also been postulated that life originated in a high temperature sulfur environment at a low pH similar to the environment that *Sulfolobus* requires for growth today.

A limiting factor in the self-assembly of early life may have been phosphorus. Phosphorus is an essential macronutrient in living organisms. It would have a significant role in the self-assembly of early life in the chemosphere [29]. One problem may have been the limiting supply of phosphorus. This would imply a mechanism may have been operating to concentrate phosphorus to a level where it was used by the assembling cells.

Deamer [19] also suggested that glyceraldehyde, hydrogen cyanide and formaldehyde could easily permeate a

membrane and serve as an energy source. He suggested that redox systems based upon hydrogen and hydrogen sulfide and the reducing potential of hydrogen gas, if an electron acceptor was present, could also be considered as possible mechanism. This in part supports the suggestion by Morita [17] that hydrogen may have been the universal energy source for early life and possibly for bacteria today during starvation survival.

Light would be able to irradiate the surface of a HM and even penetrate below the surface. Light energy was probably the most abundant source of energy on the early Earth [19]. However, to capture light, it must be absorbed by some type of pigment and then transformed to a usable form of energy. It is not known if a light-capturing pigment was present during the early stages of molecular evolution that was different from or similar to chlorophyll (which transfers electrons from water to carbon dioxide). Deamer [19] suggested some possibilities in his excellent review that included compounds such as ferrocyanide, porphyrins and polycyclic aromatic hydrocarbons. It is unlikely that a primitive pigment would have the capability of modern day photosynthetic pigment like chlorophyll. If such a pigment did exist, it would almost certainly accelerate the evolutionary process as light energy would be readily captured. The non-enzymatic synthesis of even a simple pigment is still a possibility since light was still presumably the most abundant source of energy on the primitive Earth.

2.4. Early proteins/enzymes

The first self-assembled protein would likely be composed of the most abundant amino acid(s) [30]. As less abundant amino acids became incorporated into the protein, new sequences were self-assembled. This process of protein self-assembly would lead to molecules with variations from the first ancestral protein. The first proteins would have similar sequences and as time passed, the sequences would become diverse with some possibly being catalytic as they reached a certain size and sequence.

Enzymes have two central functions: substrate specificity and catalytic efficiency. The first self-assembling cell(s) may have contained a minimal number of different enzymes that were not specific for single substrates and with slow reaction rates [31] by today's standards. Multiple substrate single enzymes may have been an advantage during early evolution. The ability of a single enzyme to catalyze numerous reactions may have been more important than reaction rates. At some time the self-assembling (self-replicating) catalytic proteins would need to fold properly from random coils into more compact structures and arrive at native states. Since there was abundant time for evolution to occur, fast catalytic rates may not have been a characteristic of the first enzyme reactions. If a catalyst is slow and has a low affinity for its substrate and product, more catalyst is required for increased product formation. However, in the assembly of the first cell(s), enzymes would only need to be catalytic and stable in the primitive cell structure, not fast and catalytic. It is also possible in the origin of life that a minimum threshold of reaction rate needed to be surpassed to allow cells to self-assemble whereas decomposition reactions could remain slow. Below these threshold reaction rates, the self-assembly of life may have been impossible. This can only be speculated upon at this time.

Sakura and Yanagawa [32] described a reaction for peptide assembly from glycine and urea in an aqueous solution. The products were *N*-carbamylglycine, *N*-carbamylglycylglycine and glycylglycine. These reactions could have provided a mechanism for the pre-biotic synthesis of peptides. If proteins were present, they may have been involved in the transition from non-enzymatic proteins to catalytic enzymes and then assembly of metabolic pathways and nucleic acids. In the self-assembly of life, the components that were available in the highest concentrations and easiest to self-assemble first would likely be used. This suggests that glycine and alanine would be initially self-assembled as they would be the most abundant and the most ancient. Other amino acids in lower concentrations would then be incorporated into the protein. This would allow variations in the sequences of the amino acids and a non-enzymatic mechanism to self-assemble new proteins in the absence of enzymes, DNA and RNA. The proteins would self-elongate and fold as they reached a certain length. The difficult and complex components such as nucleic acids would take more time and require integrated metabolism.

2.5. DNA and RNA

DNA and RNA were likely late comers in the assembly of life process. Both nucleic acids are difficult to make, requiring numerous biosynthetic steps. DNA is far from the center of present-day integrated biochemical pathways. This is also true for RNA. Reanney [33] proposed that while DNA was the high-fidelity copier or replicator system, this stability may have prevented or decreased the ability to accommodate short-term adaptations. However, RNA was more susceptible to change. Switching between these extremes of conservatism (DNA) and change (RNA) may have provided the balance necessary for the assembly of life. The mutations may or may not have any utility but at least the cells have mechanisms for selecting which mutations will occur. Also, in certain systems, information can flow from RNA into DNA, thus agreeing with the ideas proposed by Reanney [33].

Nucleic acids are chemical cousins of ATP [34]. Dyson [34] suggested an order of events possible for biological evolution. (1) RNA was present in primitive cells but it had no genetic function. (2) RNA was capable of binding to amino acids which enhanced their polymerization, permitting the formation of polypeptides. (3) The binding of

RNA to catalytic sites provided structural precision. (4) RNA binding to amino acids becomes transfer RNA. (5) RNA bound to catalytic sites eventually becomes ribosomal RNA. (6) Catalytic sites evolved from more specialized to more generalized by using transfer RNA instead of amino acids for recognition. (7) Recognition unit(s) from ribosomal RNA split off and become messenger RNA. (8) The ribosomal structure becomes unique because the genetic code takes on the function of recognition. This agrees with Cairns-Smith [22] who suggested that DNA in the early stages of evolution would be far from the center of present-day known biochemical pathways. This is also true for RNA (ribonucleic acid). Both nucleic acids are difficult to make, requiring many biosynthetic steps. According to Cairns-Smith [22], this suggests that DNA and RNA were late comers to the evolutionary process.

3. Nanobacteria and hydrogen as the universal energy source

What structure other than a very small cell (here defined as a nanocell) would be better designed for self-assembly and evolution? The answer is likely, none. It is worth considering that nanocells or nanobacteria with a minimal genome are a link between primitive cells and present-day bacteria that are ubiquitous on the Earth. Bacterial cells can be defined as open systems (both matter and energy can exit and enter; system is any part of the world or universe we define to observe and describe) as opposed to closed systems (allows energy but not matter to cross the boundary) [11]. The bacterial cell as an open system is necessary for life as we understand it. If the cell was a closed system it could not exist or evolve as there can be no novel characteristics in a closed system over time. Without change and molecular optimization, evolution would not be possible. Another aspect of a closed cell is that reactions would proceed to equilibrium (the state where there is no flow of energy across a boundary and all properties remain constant in time). If a state of equilibrium is reached in a cell, it would spontaneously degrade and die.

All nutrients must enter and leave the cell through the cytoplasmic membrane. The cytoplasmic membrane assists in the movement of charged and large compounds across the membrane by the presence of membrane transport proteins with an expenditure of energy. The bacterial cell is a remarkable, complex, open system in another way. It extracts nutrients from its external environment and uses them to grow and divide. A closed system would not be able to function in a way compatible with life as we understand it. The bacterial cell as an open system is able to replicate its DNA and divide it equally between two offspring cells.

Gases like O_2, CO_2 and N_2 can diffuse in and out of cells. Oxygen is necessary for aerobic respiration and a gas like N_2 can be reduced to NH_3 by certain bacteria, and used as a source of fixed nitrogen for metabolism. Conversely, various compounds have different permeabilities across a biological membrane. The first membranes or microspheres would have been simple by today's standards for a bacterial cell. They would need to be stable enough for molecular evolution and not restrict the entry by diffusion of simple amino acids necessary for the self-assembly of the first proteins.

Gases such as hydrogen, nitrogen, methane and carbon dioxide were present on the early Earth and have central roles in bacterial metabolism. Morita [17] suggested that H_2 is a good candidate for a universal energy source for bacteria. Many microorganisms use molecular hydrogen as an electron and energy donor and many anaerobic bacteria produce H_2. Thermophilic *Hydrogenobacter* and *Aquifex* have a membrane-bound hydrogenase. Electrons are generated and passed through an electron transport chain and ATP is generated by proton pumping and membrane-bound ATPases. It is possible that these thermophilic bacteria evolved from primitive cells and retained this hydrogenase from an ancient version of a membrane and hydrogenase enzyme that used H_2 to generate ATP.

Hydrogen utilization by nanocells/nanobacteria may have been the suitable universal energy source for the first nanocells as it was ubiquitous and can diffuse through a multitude of substances including a HM which was discussed earlier as a potential medium for self-assembly as opposed to strict aqueous medium. Bacteria still use hydrogen (protons and electrons) in oxidoreductase reactions and coliform bacteria use hydrogen as an energy source, but not for cell growth. Growth requirements are now satisfied by other complex nutrients that cells can take up in a controlled way. Nanobacteria may be the link to their primitive ancestors. The ability to obtain energy from hydrogen is slow in terms of present-day bacterial growth rates, but more than sufficient in terms of the immense time over which life originated and evolved.

4. From nanocells to present-day bacteria

This section will examine the possibility that nanobacteria are ancestors of our present-day diverse bacterial species. Moore [35] defined a cell as a membrane-enclosed entity that replicates itself autonomously and is actively growing. He also suggested that a biosphere of entirely ultra-small organisms is highly implausible and they are unlikely to exist on the Earth today. Moreover, their biochemistry would likely be a lot different from what we know today. Conversely, it is reasonable to speculate if their biochemistry is somewhat similar, but only less complex.

Folk [36] described nanobacteria as dwarf forms of bacteria with dimensions of 0.05–0.2 μm in diameter or about 1/10 the diameter and 1/1000 the cell volume of normal

bacterial dimensions. Nanobacteria have also been described as abundant in minerals and rocks and likely responsible for most of the Earth's biomass and surface chemistry on the Earth. However, the existence of nanobacteria is still under debate. Folk [36] reported that there are those who contend that nanobacteria are simply too small to contain a sufficient genome under the conjecture that a bacterial cell needs several hundred or thousand genes to survive in the natural environment. Conversely, it can be suggested that a minimal genome would not need to be very large. Velimirov [37] tries to avoid the term 'nanobacteria' which he considers ambiguous and refers to 'ultramicrobacteria'. These can be described by 13 morphological and physiological characteristics. Among them, two are especially important: (a) ultramicrobacteria show little variation in cell volume (0.05–0.09 μm^3) and cell diameter (equal or below 0.3 μm) even when exposed to high-nutrient media, and (b) ultramicrobacteria have a low DNA content (1.0–1.7 fg $cell^{-1}$).

Boal [38] speculated that a genome could be enclosed in a nanocell in the order of 100 nm (0.10 μm) in diameter with a fluid membrane to provide the cell with a boundary (an inside and outside), an interior cytoplasm at a higher pressure than the external environment and at least one informational molecule (genome). If a nanobacterial cell 100 nm in diameter is considered about 10 times smaller than a typical present-day bacterial cell, and the corresponding genome was about 10 times smaller than a present-day bacterial genome of about 4000 kb, then a minimal genome in a nanobacterial cell may be in the order of 400 kb. This 400-kb size is relatively similar to the minimal genome set. For example, 265 or 350 of the 480 protein-coding genes in the smallest known cellular genome (580 kb) of *Mycoplasma genitalium* are essential for growth and division in the laboratory [39]. It has been estimated that in the Gram-positive bacterium *Bacillus subtilis* only 9% of the current genome is necessary for growth and division [39]. The essential part of the genome was about 552 kb. This suggests that ancestral nanocells with an estimated minimal genome of about 400 kb may have been the dominant nanobacterial life form before they further evolved and diversified to present-day bacterial species. Complex genomes may have evolved from a smaller minimal genome in nanobacteria (see Fig. 1).

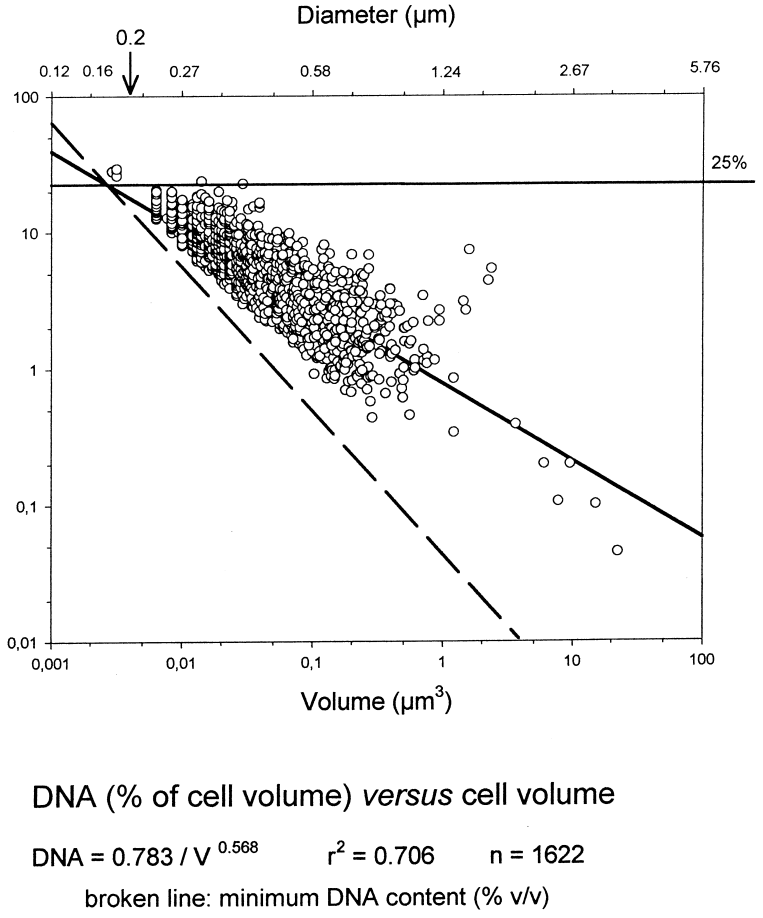

Fig. 2. Relationship between DNA and cell volume of pelagic bacteria from an ultra-oligotrophic alpine lake (Gossenkoellesee) and an oligotrophic mountain lake (Piburger See) in Tyrol, Austria. DNA was measured densitometrically according to Loferer-Krößbacher et al. [16], cell size measurements are based on image analysis techniques described by Loferer-Krößbacher et al. [15]. The spherical diameters corresponding to the respective cell volumes are given on the upper x axis.

Adams [40] suggested that the two priority factors in determining a minimal cell size are the amount of DNA needed to permit cell growth and division and the volume of the cell required to accommodate the DNA. Moreover, it needs to be determined if slow growing nanobacterial cells contain less genetic material and have limited metabolic capabilities. Adams [40] also estimated that a nanobacterial cell 50 nm in diameter could contain eight genes. A cell of 156 nm in diameter could accommodate 250 genes or about 250-kb DNA while a cell with a diameter of 194 nm could contain 750 genes.

Loferer-Krößbacher et al. [41,42] measured cell dimensions and genome sizes of extant bacteria living in ultra-oligotrophic lakes by combining fluorescent light microscopy and TEM techniques. They found cells down to a diameter of 0.2 μm and minimum genome sizes of ca. 800 kb, i.e. much smaller than most of the known (sequenced) bacterial genomes. Nonetheless, even a genome of only 500–1000 kb occupies a large portion of the volume of a cell in the size range of 150–250 nm in diameter (Fig. 2). In a recently submitted paper, however, Posch et al. [43] showed that the use of conversion factors for translating cell dimensions into biomass can lead to large errors if different staining and sizing techniques are mixed.

A definition of a minimal essential gene set required for cellular life requires an agreed-upon definition of living and what is not living [44]. A minimal gene set is theoretical as it likely does not exist in nature [44]. Moreover, a nanocell/minimal genome would require an environment in which it could exist. It is also reasonable to suggest that the nanocells require the ability to replicate their DNA, produce a corresponding message through some type of transcription and then translate that message to produce proteins. The nanocells would also require energy production and storage and a combination of passive and active transport of elements and compounds across the cytoplasmic membrane as discussed earlier in this review.

Table 1 is a summary of the cellular functions of gene classes [44]. Nanobacterial cells would require these genes to survive, grow and divide under diverse environmental conditions often less then optimal for bacteria. It is more than noteworthy that in the unknown category, *M. genitalium* contained 170 genes of which 103 were not disrupted by transposon mutagenesis and are therefore thought to be essential [44]. This means that a large number of the genes encode proteins where the function is not known. This paucity of knowledge on a simple microorganism means a good understanding of the cell and its function as a basic unit of life still awaits us in the future as more knowledge is forthcoming.

Table 1
Cellular functions of gene classes (see [44])

1. Biosynthesis of cofactors
2. Cell envelope
3. Cellular processes
4. Central metabolism
5. Energy production and storage
6. Fatty acid and lipid metabolism
7. Purine and pyrimidine metabolism
8. Regulatory
9. Replication, recombination and repair
10. Transcription
11. Translation
12. Transport (nutrients and elements)
13. Unknown

Riley [45] suggested that if ancient bacterial cells had slow growth rates, and as a consequence required fewer ribosomes, then the cells may have existed as spheres as small as 200 nm. This idea supports the nanobacteria/minimal genome cell concept which we have been discussing. It is possible that slower growing small cells that had not evolved complex integrated biochemical pathways and with the capability to use limited nutrient sources and possibly hydrogen as an energy source are present-day ancestors of very old bacteria.

If nanobacteria are confirmed to be ubiquitous and active on the Earth (and possibly in space) then experiments such as determining how viable but non-culturable the nanobacterial cells are, sequencing their genomes, studying activities (e.g. are nanobacteria capable of nitrogen fixation which would require a larger genome), membrane structure and function studies, and making bacterial artificial chromosomes in bacteria and studying functional genomics will bring forth novel information. Moreover, it would be interesting to know if they are capable of any gene transfer mechanisms and if their DNA can be used to transform normal sized bacteria. It would also be useful to know if the nanobacteria contain any extrachromosomal plasmid DNA or if their genome is more like a plasmid.

Uncertainties as to the absolute existence and link between nanobacteria and present-day bacteria suggest that the wise scientific approach is to use the scientific method and observation skills and be prepared to detect living cells over a wide range of sizes [46] and forms, and from as many diverse and unstudied samples as possible. The origin and self-assembly of life likely followed some type of sequential organization from the molecular to the biosphere level, simple to complex, chaotic/disorganized to organized and capable of growth and division. One way to achieve this may have been to self-assemble a minimal cell-minimal genome that optimized its genome and integrated biochemical machinery to a larger cell that was the basis for colonial cells and eukaryotic cells. By examining major events that most likely had to occur in a general order, it may be easier to understand the specific events that occurred during evolution. The converse is also true.

In recent times, the possibility that we are but one universe within a multiverse has been debated. This may or may not suggest that life may have self-assembled in other universes which are part of a multiverse. The origin of a

multiverse may have been from multiple big bangs as opposed to a singular event. If an event happens once, it does not mean it will happen again, especially if the events are mutually exclusive. If they are not exclusive events, then the possibility of a multiverse should be discussed and debated from an origin and evolution of life perspective and from the basis that the cell is the basic unit of life regardless of its size.

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