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Living Nanovesicles—Chemical and Physical Survival Strategies of Primordial Biosystems

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Life on Earth and Mars could have started with self-assembled nanovesicles similar to the present nanobacteria (NB). To resist extreme environmental stress situations and periods of nutritional deprivation, nanovesicles would have had a chemical composition protected by a closed mineralized compartment, facilitating their development in a primordial soup, or other early wet environment. Their survivability would have been enhanced if they had mechanisms for metabolic communication, and an ability to collect primordially available energy forms. Here, we establish an irreducible model system for life formation starting with NB.

Keywords: nanobacteria • nannobacteria • nanovesicles • extremophiles • origin of life models

Nature has stimulated the formation of biological structures allowing systems as different as NB, mitochondria, and stem cells to use a most basic energy form: light. The adaptation of the functional structure formation might have started with the NB.[1] Contemporary NB and mitochondria exhibit various elements of biological survival capabilities discovered in a large body of biological systems.[2] Especially NB and mitochondria have developed mechanisms to harvest and utilize solar energy for vital processes showing practically an equal dose, intensity, and spectral sensitivity. A closer examination of the evolutionary important light-harvesting specializations, confirmed now in mitochondria in vivo[3] and in NB in vitro,[4] has led to the design of clinically and technologically practicable strategies.[5] Here, we demonstrate that NB-like nanovesicles could indeed represent a model system for primitive biosystems due to at least seven intrinsic properties: small size, mineral protection resistant to acidic milieus, resistance to radioactivity, partial porosity of the mineral shell allowing for the passage of liquids and possibly cell signals, pumping mechanism for metabolic exchange with the environment, storage of essential biochemical components, and ability to use solar energy:

(1) A nanoscopic size, allowing for refuge in the smallest crevices and pores in crustal rocks, elevating the chances of surviving geologic events as extreme as major meteorite impacts. During transitory highly elevated Mars surface temperatures, most rocks at depths of a few meters to a few kilometers would be little affected thermally, and small microbes could most probably survive and thrive in cracks and pores.

(2) A central cavity encapsulated by a nanocrystalline apatite or carbonate shell providing some physical protection against a hostile planetary milieu subject to rapid changes, e.g., volcanic and hydrothermal activity, acidic atmosphere, and virtually planet-sterilizing meteorite impacts.[6] (Treatment with 0.1 n HCL at 20 °C for 1 day did not reduce the vitality of cultured NB, showing a higher resistance to acids than apatite from teeth.)

(3) Radiation levels on early Earth have been much higher than current levels. Thus, resistance against radioactivity was a primal necessity for the start of early forms of life. Cultured NB tolerated gamma irradiation up to a dose of 15 kGy, close to the sterilizing levels. (Irreversible inhibition started at doses of 30 kGy.)

(4) A partial porosity and selective permeability of the mineral shell are prerequisite for both the passage of liquids and cell signals.

(5) A pumping mechanism transporting nutrients and organic compounds across the mineral shell into the central cavity, and allowing metabolic products to escape, is required for growth. A possible mechanism, operating in combination with the nanoporous apatite, triggered by the diurnal solar radiation variations, is proposed below.

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(6) An ability to collect, organize, and store calcium, carbonate, and phosphate-essential inorganic components for primal biological processes. Concentrating phosphate was particularly important in the Archaean oceans with low inherent concentrations of phosphate.[7]

(7) A capability to use the solar energy for activating, accelerating, and maintaining metabolic reactions and proliferation. This ability has now been demonstrated in laboratory experiments.[4] Light energy may not be the major energy source for the primitive microbe, it may enhance or catalyze metabolic reactions based on other sources of energy.

Although still controversial, the nanoscopic carbonate precipitates associated with possible fossil microbes, revealed in meteorite ALH 84001,[8,9] could be ancient witnesses to some of these survival mechanisms (small size, ability to live in minute cracks and crevices, mineral encapsulation, storage of essential elements, etc.). Thus, the Martian and the terrestrial NB may be early life precursor forms with unique survival strategies suited for a hostile planetary environment. It is interesting that the age of the primitive Martian NB, that are associated with aqueous carbonate precipitates, has been estimated to 3.9 Gyr,[10] which closely coincides with the accepted period of giant meteorite impacts on Earth. The size of NB, with diameters from 80 to 300 nm in blood, reaching larger sizes in kidney stones, and the known, even biotechnologically exploited, specific capacity of apatite to bind proteins, nucleic acids, lipids, lipopolysaccharides, amino acids, and stains, could be regarded as the principal obstacles encountered in current attempts to isolate and identify RNA and DNA biomarkers in NB. NB possess self-propagating and self-assembling properties that have been linked with apatite.[11] However, even in view of their replication in cell-culture media,[12] NB's classification as living organisms still remains challenging, in particular because of complex membrane enclosed apatite and protein precipitates in the smallest forms of NB.[13] The study of NB's nature is additionally complicated by the rapid mineralization of the cells when calcium and phosphate are abundant in the culture media. Rapid mineralization could destroy internal cell structure and may trap or modify nucleic acid. Destruction of cellular structures leaving iron hydroxide precipitates with the gross morphology of the original cell have been traced back to rapid mineralization identified in cultures of subsurface bacteria grown in microcosms in basalt chips from the Columbia River Basalt.[14]

In addition to a protection and a calcium and phosphate storage function, spheroid apatite shells may have also performed a major biophysical key function: to collect energy from primordial sources. Apatite is a material fairly transparent to visible light with an index of refraction around 1.6. The spherical architecture of the nanocrystalline apatite shells, their size starting below the wavelength of visible light, and index-controlled waveguide properties found in tubular dentin (apatite),[15] have stimulated the formulation of an energy transfer model, based upon imaging technologies applied in near-field optical analysis (NOA),[16,17] suggesting that the apatite spheres could be instrumental in collecting and concentrating solar energy into the core of the NB. The viability of the photonic model could be confirmed under in vitro conditions: Exposed to linearly polarized white light, cultured NB responded with a uniform growth, an accelerated replication rate, and a reduced secretion of biofilm.[4] The light intensities were practically equivalent to the value of the present-day solar constant ($\sim$1000 W/m^2). The light doses employed were at levels around

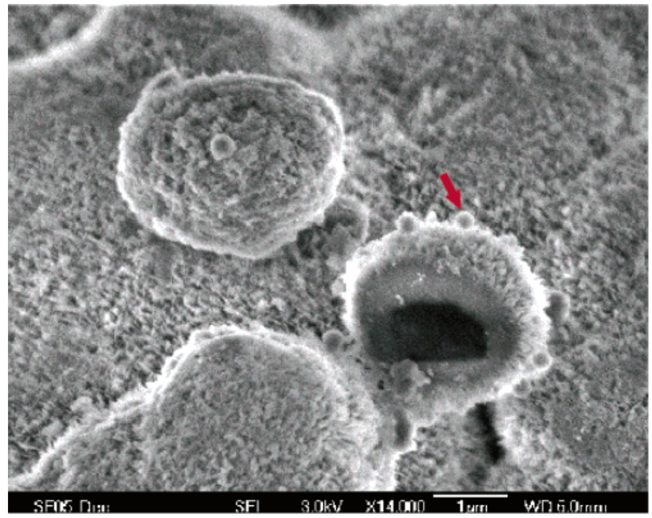

Figure 1. Three giant nanobacteria formed in serum-free media (DMEM) within one month culture period, SEM-image, bar 1 μm. The equiradial spherical satellites (Ø $\approx$ 200 nm) attached to the apatite igloo ($\rightarrow$ small arrow) are presumably nanobacteria liberated from one mother cavity in which they matured together.

4×10^4 Jm^{-2}. Equal light doses and intensities have been reported to induce biostimulatory effects in numerous biosystems, in vitro and in vivo,[18] and to elevate the survival level of various stress exposed cells, including stem cells,[2] neuronal cells,[19] and retinal cells.[20] While the physical properties of the apatite shell may provide an excellent mechanism for harvesting solar energy—the principal primordial energy form on Earth—metabolic and energy transfer routes from NB to the biosystems symbiotically hosting the NB, seem also plausible, in particular because the apatite coating promotes NB internalization into cells.

Low level laser light of an intensity of 1000 W/m^2 has been demonstrated to modulate the thickness of nanoscopic water layers attached to translucent polymer films,[21] presumably inducing transient liquid–vapor phase transitions. Due to the light-focusing function of the spheroid apatite shell, and the permeability of the porous shell for fluids, we could assume that light intensities of the order of 1000 W/m^2 will induce powerful molecular flow processes, potentially pumping water and suspended components from the central cavity (Figure 1), across the porous shell, to the external side (Figure 2). Regulatory processes reflecting the permeability of the apatite shell have been observed in cultured NB, responding spontaneously to their exposure to stress by secreting large amounts of mucus in short periods of time.[24]

By assuming the mineral cavity to provide a setting for the interplay between light, apatite, water, and RNA, NB advance to a functional biochemical platform for the self-assembly of early living nanocells. This interplay could be enriched by an interfacial transfer of biomolecules between donor and recipient biosystems, promoted by long-term physical proximity and release of connective mucus, a process similar to the horizontal transfer of genetic material verified in bacterial conjugation.[22,23] Interfacial mass transfer between contacting biosystems is believed to be a stress-induced phenomenon of prime evolutionary importance, securing the formation and the survival of bacterial and NB colonies.[24,25]

Inhibitory effects were achieved in NB by the administration of several antibiotics.[24] In particular, cultured NB were irrevers-

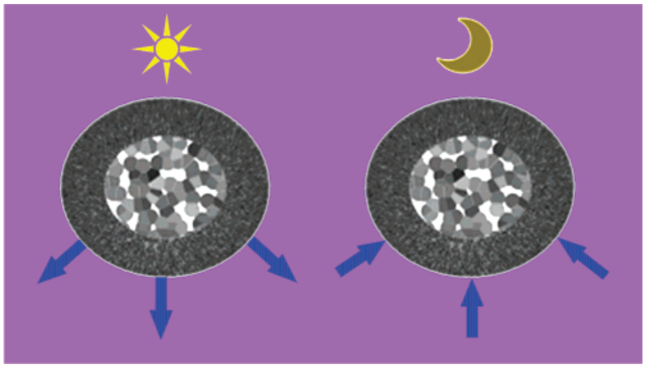

Figure 2. Artist's view of the light effect on nanoscale biosystems coated with apatite: Irradiation and differential heating with light intensities of the order of the solar constant causes fluid outflow via the porous apatite shell (left). Darkness reverses the processes in the cavity, causing inflow of fluids and nutrients. Light-induced diurnal temperature variations may have a "natural thermocycler" effect on nucleic acids.

ibly inhibited by a temporally extended exposure to tetracycline. Tetracycline is known to inhibit ribosomal protein synthesis and catalytic RNA functions, a paradigm in the origin of life concepts. Assuming that primitive NB contained RNA, transient exposures to compounds inhibiting RNA functions, potentially present in a primordial environment, could have been efficiently shielded by the specific binding properties of the apatite capsule. Tetracycline is representative as an inhibitor of catalytic RNA functions. Indeed, cultured NB were not susceptible to transient exposures to tetracycline, although were inactivated after long-term exposure.[24]

The responses to the external stimuli indicated that NB represent some form of life specialized to survive extreme environmental conditions. This is realized via a functional balance between the synergistic interaction of physical and chemical properties, utilization of natural energies, and maximum possible protection. A biosystem unifying all these qualities seems of paramount importance for a biological development in a primitive planetary world. Parts of this basic interplay might apply to photobiological mechanisms observed to occur in mitochondria, and various tissues known to respond to photostimulation with visible light when the light dose and the intensity were equal to the parameters used in irradiating NB.[5,26] Coexisting with mammalian cells,[27,28] NB may possibly represent first life forms still existing on Earth. By linking physical and chemical processes found to be related to life-involving phosphorylated metabolites and building blocks, they may ultimately lead us to a better understanding of the mechanisms by which nanoscale cells could function and replicate in a uniquely simple manner, relevant for early life forms, and for novel methods in the design of nanovesicles mimicking biosystems.

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