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Nature creates, adapts, protects and sustains life using hydrogen sulfide

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1. ABSTRACT

Life emerged on Earth in an anaerobic environment, bathed in noxious gases. Among these gases, the role of hydrogen sulfide is significant since this gas, was required as a building block of life, contributed to abiogenic generation of organic compounds that gave rise to life and drove adaptations of life throughout its entire evolutionary path. During evolution, hydrogen sulfide contributed to sustaining life in face of harsh environmental conditions. Modern cells still utilize hydrogen sulfide as a signaling molecule, in pro and anti-inflammatory responses, for acquisition of tolerance against damage, in directing repair responses, as a source of energy and in modifying their genetic makeup and function to acquire a phenotype reminiscent of early life forms.

2. LIFE FORMS IN PRESENCE OF HYDROGEN SULFIDE

The idea that all organisms emerged, through random variation and natural selection, from a common ancestor, was first made in the 1740s by Pierre-Louis Moreau de Maupertuis and subsequently by Charles Darwin (1-3). In his book, "On the Origin of Species", Charles Darwin stated that "Therefore I should infer from analogy that probably all the organic beings which have ever lived on this Earth have descended from some one primordial form, into which life was first breathed" (2). Darwin envisioned that life arose from non-living matter, a process now known as abiogenesis, as a "warm little pond, with all sorts of ammonia and phosphoric salts, lights, heat, electricity, *etc.* present, so that a protein compound was chemically formed ready to undergo still more complex changes". Consistent with the idea of a common ancestor, the rate of genetic divergence between species or between populations within a species has shown that populations that are closely related have many similar genes, have small genetic distances, and have a recent common ancestor (4). It is now thought that approximately 750 million years after Earth was formed, simple organic compounds formed life by a process of biogenesis that ultimately gave rise to a common progenitor, the last universal common ancestor (LUCA) or cenancestor. All life forms evolved from this ancestor that is estimated to have lived about 4.4-4.2 Ga ago (4-7). Phylogenetic trees that were constructed based on protein, DNA, or rRNA sequences have given credibility and firm quantitative support for existence of a common ancestor of life (8-9).

Life on Earth was created only when a solid crust was formed and there was water where chemical reactions could take place and complex organic matter could form and evolve. The initial Earth was in-hospitable and is believed that life got started in hot and reduced environments. In fact, there are many extremeophiles that still live today in the harshest conditions on Earth. Among these, are thermophiles, which live in areas of high temperature such as hot springs and around volcanoes, halophiles that live in areas with a high salt content or low water, and methanogens, that live in poorly aerated swampy areas. The macro-molecular

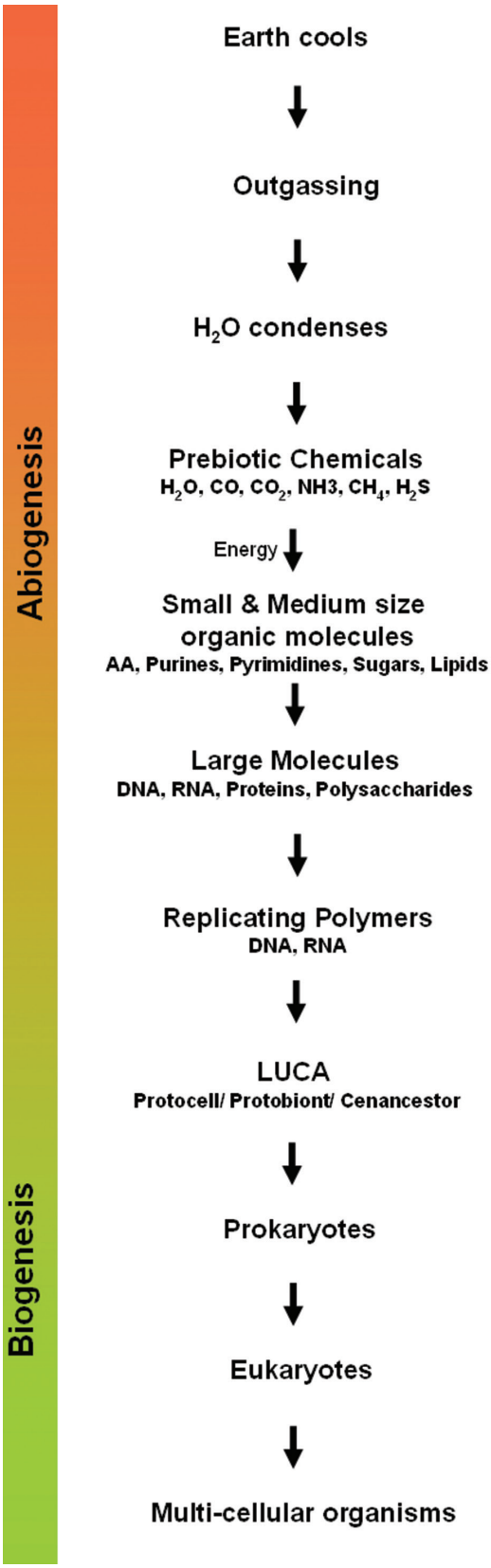

Figure 1. Formation of life on Earth from abiogenesis to biogenesis.

constituents of these Prokaryotic microorganisms closely resembles those which are present in Eukaryotes. In fact, now besides the bacteria and the Eukarya, these microorganisms form the third taxonomic superkingdom or 'domain'. However, the precise steps that led to the formation of life are not yet fully understood, nor it is clear whether this process took place on early Earth or originated elsewhere and then arrived on Earth by comets and asteroids. Some even suggest that the complex organic molecules that gave rise to life were initially formed in the dust that surrounds our early sun or other stars and then was brought to Earth by asteroids, comets or meteorites (10).

Life got started as abiotic chemical synthesis of organic compounds that gained complexity and new properties over time (11-12) (Figure 1, Table 1). One property that is a pre-eminent feature of life is to generate energy and store certain information such as encoding useful reactions of organic compounds. Another requirement is that the encoded information should be sustained through a process of self-replication. The initial step in the abiogenesis required self-replicating or at least duplicating molecules. Thus, biogenesis first requires abiogenic generation of biopolymers that can transfer and duplicate the reactions that are encoded within them. These initial reactions might have had a hot start in early Earth in a reducing atmosphere rich in methane, ammonia and water vapor or occurred under extreme conditions such as around vents of hot volcanoes.

There are several competing theories for the origin-of-life and the direction of chemical reactions that occurred in early Earth. According to the metabolism-first theories, the initial chemical reactions might have driven metabolism which started with simple molecules that built increasing levels of complexity. Proponents of replicator-first theories suggest that the initial reactions gave rise to simple organic molecules that became competent to self-replicate. These compounds could be closely related to DNA or according to the "RNA World Theory," were self-replicating RNA species (13) (Figure 1). It has been suggested that nucleoside bases and sugars were formed first. This was followed by formation of prebiotic nucleotide bases, sugars, and inorganic phosphates or polyphosphates, which accumulated in an adequately pure state (Table 1). The interaction of these compounds with montmorillonite presumably generated long single-stranded polynucleotides. Some of these polynucleotides were converted to double strands by a template-driven synthesis leading to accumulation of double-stranded RNAs in the primitive Earth (14) (Figure 1).

Despite the theories that postulate that life emerged from specialized complex molecules, such as RNA, more recent evidence as well as computer simulations suggest that life might have got started without use of biopolymers. The early constituted systems might

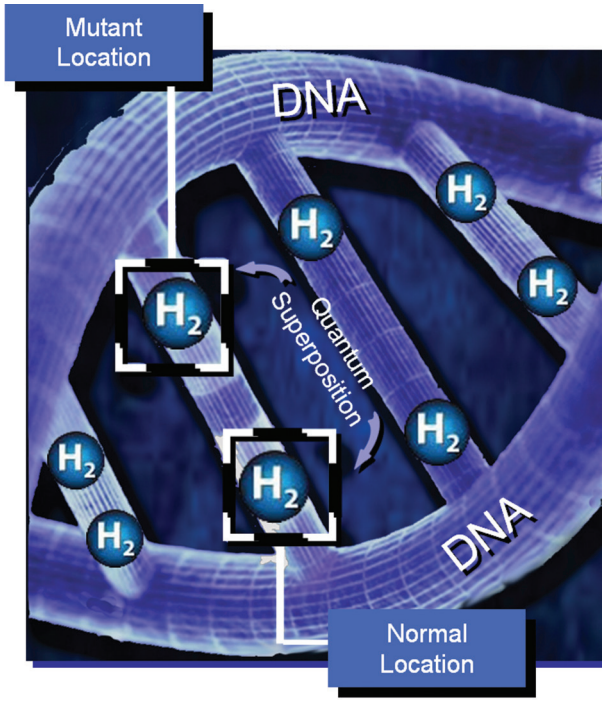

Figure 2. Quantum superposition of hydrogen atom on DNA strand gets fixed in normal or mutant location by cues in the environment. Modified from (20).

Table 1. Organic compounds formed in prebiotic conditions

Amino acids	Carboxylic acids	Nucleic acid bases	Sugars
Glycine	Formic acid	Adenine	Pentoses
Alanine	Acetic acid	Guanine	Hexoses
Valine	Propionic acid	Cytosine	Ribose
Leucine	Fatty acids	Uracil	
Isoleucine	Glycolyic acid	Xanthine	
Proline	Lactic acid	Hypoxanthine	
Aspartic acid	Succinic acid		
Glutamic acid			
Serine			
Threonine			
α-Aminobutyric acid			
Modified from (11)			

have been comprised of non-covalent proto-cellular assemblies, capable of storing information, and exhibiting inheritance and selection, and catalyzing recruitment of diverse amphiphilic and hydrophobic compounds. This was followed by a series of complex chain of evolutionary events, that only subsequently, led to the appearance of DNA, RNA, proteins and enzymes and creation of the common ancestor of all living cells (15).

According to the Darwin's theory of evolution, environment selects random mutations and other DNA changes that adapt species to their environment. However, gradual evolution is seldom seen in the fossil records and new species appear at random intervals. The existing fossil records of evolutionary progression show that most species suddenly appear, and in many cases close to a million years later, without any change in their external appearance, disappear. These rapid periods of evolutionary change have been interrupted by periods of evolutionary stasis (16.-17). Geological records also show that, on very rare occasions, entire new families, orders, and classes of organisms developed rapidly (18). Local adaptation occurs only on the scale of decades and tens of meters. Adaptation stipulates that genes actively adapt to the passive environment rather than the environment acting to elicit change in an otherwise passive gene. It appears that the standard evolutionary theories can not adequately explain the extremely rapid evolution of mutation rate, genome size, and chromosome structure (19). Thus, some have proposed that evolutionary changes might be forcing such adaptations by directly interacting with DNA. One mechanism for these cell driven mutations might be initiated by quantum events such as by the shift of a single proton (hydrogen atom) from one strand of DNA to an adjacent site on the opposite strand of DNA. Quantum coherence, which is described as both the shifted and unshifted protons, can be destroyed by the process of decoherence, in which the quantum state of the genome gets entangled, for example, by the use of a substrate within its environment (20) (Figure 2). Some consider that '...the selective generation of mutations by unknown means is a class of models that cannot and should not, be rejected' (20).

What are the environmental factors that deeply impacted evolution from its inception? Ranking only second to water, gases in the primitive Earth, have probably played a significant part in generation of organic compounds that led to formation of life. Among these, hydrogen sulfide has played a significant role in abiogenic processes that formed various complex organic compounds (Figure 3). As discussed below, this gas also shaped the evolution of many life forms on Earth though mass extinctions as well as by integrating into regulation of a variety of cell functions including metabolism, stemness, damage tolerance, repair, growth, and longevity. Numerous studies have shown that many of the simple and complex organic matters that participated in creation of early life forms were formed in presence of hydrogen sulfide and other sulfur containing compounds.

The first description of hydrogen sulfide can be traced back to the 15th century when Johann Baptista

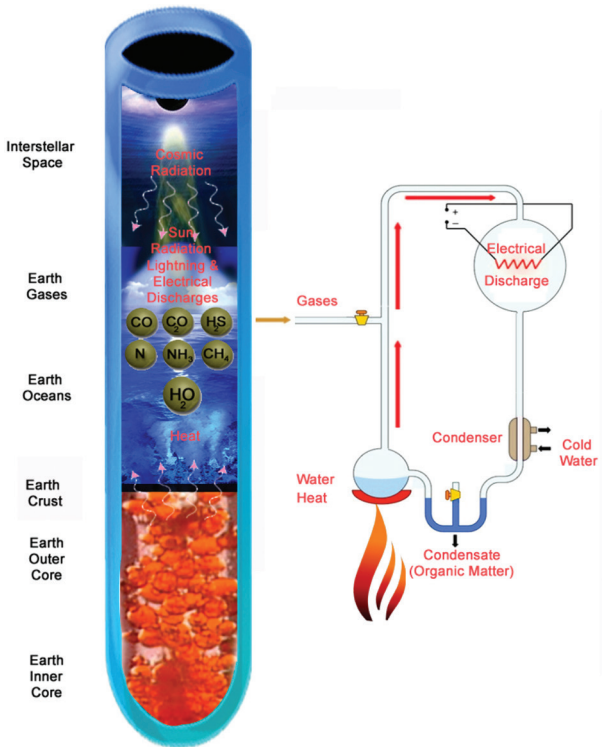

Figure 3. Life forming conditions on Earth and apparatus of Urey-Miller simulating these conditions (12,244).

van Helmont (1579-1644) described hydrogen sulfide to be part of hepaticus (hepatic air) or one of the gases of putrefaction (21). The first person who described the toxicological effect of hydrogen sulfide is likely the Italian physician, Bernardino Ramazzini (22). The chapter entitled, "Diseases of Cleaners of Privies and Cesspits," describes the health effects of hydrogen sulfide in privy and cesspit workers. Ramazzini comments on the effects of hydrogen sulfide on the workers' eyes "…those foul exhalations wage ruthless war, and they attack so cruelly with their piercing stings that they rob them of life, that is to say of light." Hydrogen sulfide was first synthesized by Carl Wilhelm Scheele who identified that the smell of rotten eggs is due to the release of the hydrogen sulfide (21). Interestingly, French writer Victor Hugo, in his famous 1862 novel, Les Miserables, described hydrogen sulfide as a horrid, smelly waste that was actually the life-blood of the people of his city (23).

The principal components of volcanic gases are water vapor (H_2O), carbon dioxide (CO_2), nitrogen, argon, helium, neon, methane, carbon monoxide and hydrogen. Volcanic eruptions emit into the atmosphere, not only nitrogen, hydrogen, argon, helium, neon, carbon monoxide (CO), carbon dioxide (CO_2), and methane (CH_4), but also sulfur either as sulfur dioxide (SO_2) (high-temperature volcanic gases) or hydrogen sulfide (H_2S) (low-temperature volcanic gases). Once the water on Earth condensed into oceans, the atmosphere of Earth was essentially stripped away from its sulfur gases and these gases, because of their high solubility in water, enriched levels of hydrogen sulfide in the oceans. Also, the gases released from volcanic eruptions, warmed the oceans, and lowered the capacity of the water to absorb and retain oxygen. The lowering capacity of warm oceans to retain oxygen, in turn, led to an increased level of hydrogen sulfide in oceans that is normally oxidized by oxygen. Thus, in many niches on Earth, early life forms were created and then were exposed to an elevated level of hydrogen sulfide. Not only on Earth, but the interstellar medium, is rich in Hydrogen sulfide and this gas contributes to much of the sulfur in carbonaceous meteorites (24-26).

The idea that life emerged from abiotic generation of complex organic compounds is supported by many lines of evidence. Aleksandr I Oparin formulated the hypothesis that the geochemical formation of cellular constituents and formation of life on Earth occurred in the reducing environment of the early Earth (27-29). There is a general agreement that the atmosphere of early Earth was poor in oxygen and was likely weakly reducing, and contained NH_3, N_2, CO_2, H_2O, CO and lesser amounts of more reduced gases such as H_2S, CH_4, and H_2 (30). Much of the atmospheric hydrogen sulfide of the early Earth was likely derived from volcanic eruptions which subsequently was continuously depleted from the Earth atmosphere (31-34).

The abiotic synthesis of organic compounds under conditions that simulates the primitive Earth atmosphere has given credibility to the idea that hydrogen sulfide is essential to formation of early life forms (29,31,35) (Figure 3). Some organic matter in early Earth might have formed through reactions between CO and H_2 in presence of NH_3 (36). Yet, others have considered that organic synthesis might have proceeded without the need of a highly reducing atmosphere that contained CH_4 or H_2 (37). For example, an irradiated mixtures of CO, CO_2 and H_2O in presence of silicates produced organic matter including formaldehyde, formic acid, acetaldehyde, and glycolytic acid. A mixture of H_2, H_2O, CH_4, and NH_3, a composition commonly present on solar system bodies (e.g. protosolar nebula, ancient Mars, Titan) and also being present at least locally or transiently; in the early Earth, yielded organic compounds. Upon applying energy in the form of high voltage electrical sparks simulating lightning discharges, which frequently appear during volcanic eruptions, the gas mixture yielded, the non-biological, non-enzymatic and simultaneous synthesis of the amino acids, alanine and glycine. In similar studies, discharge of electric discharges into a mixture of H_2, CH_4, and NH_3, in presence of water, led to the formation of a variety of organic molecules, including several amino acids. However, when hydrogen sulfide was added to the gas mixture, virtually all proteogenic amino acids including

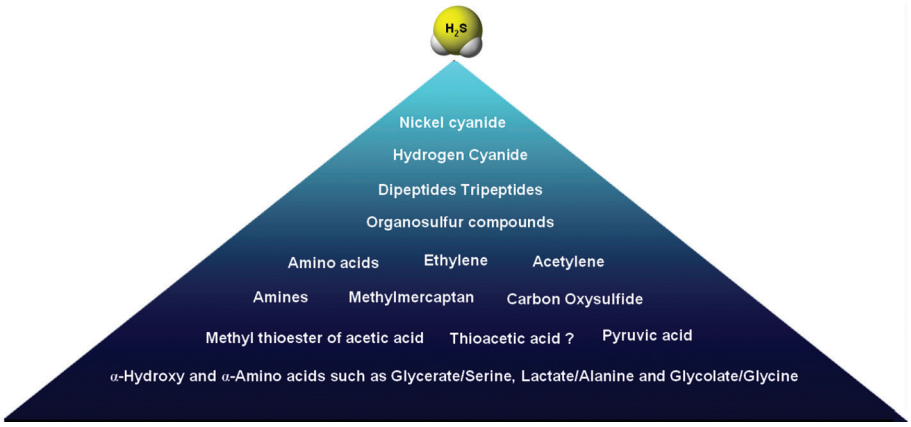

Figure 4. Compounds formed in presence of hydrogen sulfide.

a total of 23 amino acids as well as 4 amines, and 7 organosulfur compounds (S-methylcysteine, methionine, ethionine, methionine sulfoxide, and methionine sulfone) were formed (38) (Figure 4). With a gas mixture of CH_4, C_2H_6, NH_3, and H_2S used as a photosensitizer for long-wave UV radiation, alanine, glycine, serine, glutamic acid, aspartic acid, and cystine were produced (39). Significant yields of methionine were also reported from the action of an electric discharge on a mixture of CH_4, H_2O, N_2, NH_3, and H_2S (40). Others report that when H_2S was added to other reduced gases, including CH_4, and NH_3, and these were subjected into a lightning-rich atmosphere, hydrogen cyanide, ethylene, and acetylene were formed which can then serve as intermediate chemicals required for the formation of more complex prebiotic organic compounds (41) (Figure 4, Table 1). Although one possibility is that such organic compounds were formed on Earth, equally possible is that they were first formed elsewhere and then were brought to Earth. Since complex organic molecules, including RNA precursors are relatively common in the solar system and interstellar space, such compounds might have participated in the abiogenesis on Earth (42). There is some evidence that clay might also have played a role in creation of organic compounds. For example, both activated and non-activated amino acids can be generated on clay and it is suggested that polymerization of amino acids, nucleotide bases and other macromolecules might have occurred on clay or phyllosilicates (43-47).

Some consider that early life forms, capable of reproduction, emerged at c. 4.2 Ga from iron monosulphide bubbles at a submarine hydrothermal front where hot (c.150°C), alkaline, extremely reduced flow, rich in bisulphide, entered the warm (c. 90°C), acid and iron rich Hadean ocean. Based on this hypothesis, synthesis of organic anions occurred on nickel laced FeS membranes which acted as a semipermeable catalytic boundary between the two fluids. The water of oceans provided carbonate, phosphate, iron, nickel and protons and the hydrothermal solution provided the required ammonia, acetate, HS^-, H_2 and tungsten, as well as minor concentrations of organic sulphides and perhaps cyanide and acetaldehyde. The generation of organic anions would have led to an increase in osmotic pressure within the FeS bubbles, driving distension, budding and reproduction of the bubbles similar to and as the first step towards cell division (48).

One of the early steps towards the genesis of life, has been best described by the iron–sulfur world theory offered by Wächtershäuser. Based on chemical experiments and extant biochemistry, he retrodicted creation of early “pioneer organisms” that were formed on the surface of iron sulfide minerals at a high pressure and temperature at the hydrothermal flow of volacanic eruptions (48). Although, these organisms were not able to divide, and despite the fact that they did not have enzymes or a translation machinery, they were able to grow and had an autocatlaytic two dimensional surface metabolism confined in a mono-molecular organic layer. These organisms had a composite structure of a mineral base with transition metal centers that catalyzed generation of small non-polymeric organic compounds from inorganic gases such as carbon monoxide, carbon dioxide, hydrogen cyanide and hydrogen sulfide, which are the same gases that exist in the volcanic plumes. The hydrogen sulfide is essential, not only as an electron donor, but in acting as a nucleophilic catalyst. These organic compounds represent the first autocatalytic “surface type of metabolism”. By their own retention on or in the mineral base as organic ligands, they amplified their own production, a representation of the first step towards self replication. This led to the formation of an initial metabolic pathway comprised of primitive sulfur-dependent version of the reductive citric acid cycle in modern cells. This metabolic pathway has evolved by generation of accelerated catalysts and subsequently by the formation of new metabolic products that further accelerated such catalysts.

It is now demonstrated that, as it occurs in volcanic eruptions, mixing ferrous sulfide with pyrite (FeS_2) using hydrogen sulfide as a reducing agent leads to formation of FeS_2, $2H^+$(or H^2) and $2e^-$ (49-50). Ferrous ions are ubiquitous, hydrogen sulfide has been abundant in early Earth, and pyrite formation requires anaerobic conditions which is compatible with the geochemistry of early Earth. Methylmercaptan and carbon oxysulfide can be generated from CO and H_2 in the presence of NiS and from a mixture of CO_2 and FeS, in presence of H_2S (51-52). It is also suggested that prebiotic formation of ammonia occurred from dinitrogen on iron sulfide surfaces. In such a reaction, presence of H_2S is required as a reductant (53). A mixture of carbon monoxide and hydrogen sulfide, in presence of nickel and iron sulfides, led to generation of simplest activated acetic acid a nalogues such as methyl thioester of acetic acid and presumably thioacetic acid. These compounds formed the foundations for creation of metabolic pathways that ultimately led to generation of acetyl-CoA by modern cells (54). The hyperthermophilic, chemo-litho-autotrophic origin of life, in an iron-sulfur world also served in the formation of phospho-anhydride compounds as well as in coupling the energy production with endergonic reactions (55). Thus, the first organisms with surface type of metabolism are characterized as catalysts that fostered the generation of pyrite by providing a catalytic pathway for the flow of electrons from hydrogen sulfide to carbon dioxide.

Following the development of two dimensional metabolists, the stage was ready for a second wave of evolution. This evolutionary step led to acquisition of an auto-trophically grown lipid membrane to create semi-cellular organisms that were still supported by a mineral surface and had both a membrane and cytosolic metabolism (55). Wächtershäuser describes the conditions that still exists and which are habitats where life is formed as “a place with liquid water having a nearly neutral pH and high salinity; a place with a high temperature and a high pressure; a place where hydrogen sulfide, carbon dioxide, and nitrogen are pressured into reaction in the presence of ferrous and other catalytic metal ions; a place where hot volcanic exhalations clash with a circulating hydrothermal water flow; a place deep down where a pyrite-forming autocatalyst once gave, and still is giving, birth to life” (55).

In presence or absence of ferrous hydroxide, hydrogen sulfide or methyl mercaptan, nickel hydroxide reacts with hydrogen cyanide to generate nickel cyanide along with significant quantities of pyruvic acid. In turn, the reaction of nickel cyanide with carbon monoxide generates pairs of α-hydroxy and α-amino acids such as glycerate/serine, lactate/alanine and glycolate/glycine. In presence of ferrous sulfide, pyruvic acid or other α-keto acids can react with ammonia and hydrogen sulfide to generate alanine or other α-amino acids (56). Once α-amino acids interact with carbon oxy-sulfide or with CO and hydrogen sulfide, the reaction generates a peptide cycle that fosters formation of dipeptides, tripeptides, *etc*. These are subsequently degraded via hydantoin and urea moieties which cause the cleavage of the amino acid at the N-terminus of the peptide (54,56-57). The frequency that amino acids are incorporated into proteins has evolved during evolution. For example, the sulfur containing amino acid, cysteine, which is utilized to produce hydrogen sulfide, is under-represented in the LUCA proteins, relative to their modern descendants. Analysis of extant proteomes from over three Ga ago has revealed that the use of this amino acid in proteins was substantially increased in frequency only after divergence of the three primary cell lineages (58).

In an attempt to understand the intermediate stages of abiogenesis, Sidney W. Fox, based on studies in 1950s and 1960s, simulating the conditions of early Earth, proposed that peptide structures or proteinoid microspheres were the precursors to the creation of proto-cells or protobiont (59). According to Fox, these predecessors to living organisms were microspheres or protein proto-cells, which were self-organized, endogenously ordered, spherical collection of lipids. Similar to cells, they had an outer wall, were sensitive to osomolarity of their surroundings and could divide. Protenoids are formed when amino acids are allowed to dry even at low temperatures such as at 70^o C which mimics conditions present in the prebiotic dry spots. These structures are formed since some of the amino acids form peptide chains are more hydrophobic which allows them to form microdroplets. Although, these initial studies were the catalysts to other theories on emergence of life, certain aspects of these microspheres are not consistent with those that are seen in cells. Proteinoids are not, in a true sense proteins since they have non-peptide bonds and cross linkages that are not present in proteins of living cells.

In the “prebiotic chemistry” of life, complex organic molecules could freely interact and combine until they got segregated from their environment. The formation of microdroplets produced a state of molecular isolationism and forced the segregated molecules to stay and evolve together. These droplets had two attributes; they not only could replicate through fission, they also could fuse with other droplets (28). Although Oparin suggested that metabolism preceded the replication, the question as which one occurred first is not yet settled. The first living cells might have arisen by polycyclic aromatic hydrocarbons (PAH), and the enclosure of self-replicating RNA in a membrane comprised of phospholipids which are the basic components of all present day biological membranes, including the plasma membranes of both prokaryotic and Eukaryotic cells (60).

To replicate how life was formed, many are attempting to produce artificial cells; living cells that are

synthesized to contain macromolecules including those that store information and have the potential to divide or mutate, capture energy, and maintain ion gradients (61). Although such an idea is not yet fully achieved, a cell that its genome was entirely synthetic has been produced, yet this cell, in a true sense, is not completely artificial since it uses the preexisting cytoplasmic components that were previously present in the cell (62). Using simple organic and inorganic compounds, it was reported that cell-like structures called Jeewanu or particles of life, which are reminiscent of primitive life forms, can be synthesized of semipermeable membrane, amino acids, phospholipids, carbohydrates and nucleic acid bases that form DNA and RNA. Although, these structures, similar to some organisms, showed budding, they lacked the ability to grow and divide (63). For these reasons, their true nature as it relates to living organisms has been debated (64). Additional studies showed that structures that contain RNA like materials can be formed that show metabolic activity and grow from within (65).

New technologies are being developed to direct evolution by generation of artificial genetic codes. This is achieved by virtue of incorporating un-natural nucleotides in DNA that code for alternative amino acids and by extending the normal repertoire of amino acids beyond those that are utilized by the 61 codons that code for the known amino acids (66-68).

3. HYDROGEN SULFIDE DROVE THE PATH OF EVOLUTION

Sulfur moiety and hydrogen sulfide have been omnipresent on Earth, and persistently impacted the evolution of life on Earth. Because of ability of sulfur to exist both in reduced ($^{2-}$) and oxidized states ($^{6+}$), its usage started in prebiotic Earth and still continues in modern cells. In hydrogen sulfide rich environments, some microorganisms adapted to use hydrogen sulfide as a source of energy and/or for generation of other organic substances, while other organisms started to synthesize it by modifying organic matter. These microorganisms, were created, resided and flourished around the volcanic eruptions on the Earth surface or within the ocean floor. The earliest fossil evidence of life dates back to 3.5 to 3.8 Ga ago (4-5). These fossilized early life forms, which resemble bacteria, are elongated hollow tubular cells, that form chains and coat grains of sand. Sulfur metabolism evolved quite early since close to the root of the tree of life, there are numerous bacteria that metabolize sulfur species. These include microorganisms which rely on generation of energy from dissimilatory elemental sulfur reduction, dissimilatory sulfate reduction, or anoxygenic photosynthesis (69). Similar to many of the deep branching bacteria of the sulfur cycle that could survive at very high temperatures (hyperthermophiles) in the early Earth, organisms are commonly found in present-day sulfide-rich hydrothermal systems (69). However, there are various lines of evidence that indicate that photosynthesis developed earlier than sulfate respiration. Also, since oxygen-respiring multicellular Eukaryotes appeared later in the Precambrian, it can be deduced that sulfate respiration must have antedated oxygen respiration (70). Anoxygenic photosynthesis as well as the first evidence for sulfate reduction was established by 3.5 Ga, and the first evidence for oxygen production by oxygenic photosynthesis is found at around 2.8 Ga (69). The Archaea, Bacteria, and Eucarya domains show common properties that indicate that earliest life forms were reliant on inorganic nutrition. Photosynthesis and the use of organic compounds for carbon and energy metabolism occurred later during evolution (71). The ability to metabolize sulfur compounds is widespread throughout the Bacteria and Archaea domains. These organisms reduce elemental sulfur to hydrogen sulfide, utilizing both H_2 and organic compounds as the electron donor (69). In the anoxic Earth, early life forms carried out photosynthesis and could oxidize sulfur as an electron acceptor in the mineralization of organic matter, completing the carbon cycle (72). Bacteria that process sulfur still exist beneath the surface of the sand of today's beaches and in sulfidic environments within the oceans.

The gases of the early Earth and the organic matter that resulted from these gases are among the substances that have been used by nature to force organisms to become tolerant to their surroundings, likely, by becoming dependent on such matters or by forcing those that remain to further generate such gases. Among the mechanisms that changed evolution is the sulfate concentration of oceans. The levels of sulfate in seawater remained below 1 mM, and did not increase to >1 mM until around 2.3 Ga. Sulfate levels increased slowly, and by 1.8 Ga, sulfate concentrations increased sufficiently to increase rates of sulfate reduction. It is proposed that the oceans remained sulfide rich until the Neoproterozoic. At around this time, there was an increase in the levels of atmospheric oxygen to >10 percent of its present-day levels. Such an increase promoted the widespread oxidation of marine surface sediments and led to an evolutionary divergence of sulfide oxidizing bacteria (69).

Hydrogen sulfide might have played a significant role in selection of organisms that could live in sulfidic rich environments. For example, the analysis of organic residues from mass extinction boundaries has revealed that the oceans were anoxic and yet they had planktons that metabolized H_2S (73). The microorganisms that remained included those that relied on hydrogen sulfide as the source of their energy. A group of microorganisms that might have resisted hydrogen sulfide induced extinction might have included those that through their metabolism yielded hydrogen sulfide themselves. Such bacteria are capable of reducing elemental sulfur or by using sulfur oxygenase and sulfite oxidase enzymes, can oxidize inorganic sulfur compounds, such as sulfite, or

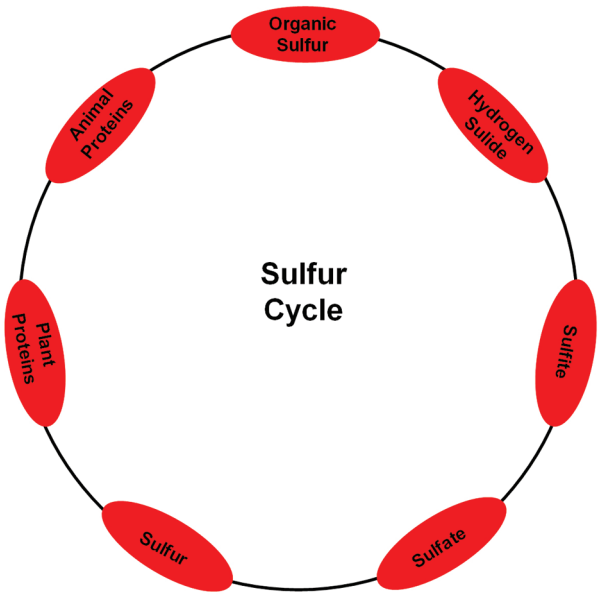

Figure 5. Sufur cycle on Earth.

thio-sulfate to hydrogen sulfide. For example, based on the differential toxicity to sulfide of PS (photosystem) II and PS I, four different types of adaptations to sulfide have been identified in cyanobacteria. These include bacteria that carry out sulfide dependent anoxygenic photosynthesis, or only carry out sulfide insensitive oxygenic photosynthesis. Yet, some cyanobacteria are capable of carrying out sulfide insensitive oxygenic photosynthesis as well as sulfide dependent anoxygenic photosynthesis (74).

The early life forms started the sulfur cycle around 3.5 Ga ago (69,75-76). This cycle involves either incorporation of sulfide into organic compounds or conversion of organic sulfur back into inorganic forms (Figure 5). The inorganic forms include hydrogen sulfide (H_2S), sulfide minerals, sulfite, and elemental sulfur, which are oxidized to sulfate (SO_4^{2-}) or are reduced back to sulfide. Other sulfate-reducing bacteria reduce a variety of sulfur containing substrates including dimethyl sulfoxide (DMSO) (77). Many sulfur reducing bacteria and Archea derive their energy by reducing sulfate (SO^{2-}). While some bacteria reduce small amounts of sulfates in the so-called assimilatory sulfate reduction, others, for production of energy, utilize dissimilatory sulfate reduction that uses large amounts of sulfates as the terminal electron acceptor of the electron transport chain (75). Together, these sulfate-reducing microorganisms have blossomed throughout the evolution to include 60 genera, and encompassing 220 different species (75). The use of H_2S in metabolism has remained conserved throughout the evolution since many modern species of microorganisms including mammalian cells metabolize H_2S.

Some lithotrophs, in a process called chemosynthesis, use sulfur compounds to produce sugars, while, in a process similar to photosynthesis, other bacteria and Archaea use hydrogen sulfide as the electron donor and oxygen as the electron acceptor in production of sugars and yet others such as green and purple sulfur bacteria oxidize hydrogen sulfide to produce elemental sulfur. Thus, these microorganisms breathe sulfate in a fashion that releases them from being reliant on oxygen for generation of energy (78).

Besides bacteria, the primary producers of organic sulfur compounds are certain plants that have coupled photosynthesis to the reduction of sulfate ion. This is achieved by converting sulfate ion to sulfide and assimilating the sulfur into cysteine, from which most other organo-sulfur compounds such as cystine, and glutathione are synthesized (Figure 6). Prokaryotes, fungi, and plants synthesize methionine from cysteine using the enzymes, CGS and CBL, while production of hydrogen sulfide in plants is mediated by cysteine-synthesizing and degrading enzymes, such as O-acetyl-L-serine (thiol) lyase(OAS-TL) (EC 4.2.99.8), L-cysteine desulfhydrase (EC 4.4.1.1), and 3-mercaptopyruvate sulfurtransferase (EC 2.8.1.2) (79) (Figure 6-7). Besides plants, invertebrates such as Manila clam *Tapes philippinarum,* the lugworm *Arenicola marina* and the worm *Urechis caupo Fisher* produce significant quantities of hydrogen sulfide gas (80). Macrofauna, which are benthic or soil organisms on the ocean floor, and which are generally comprised of sedentary invertebrates such as bivalves, tube worms, and some burrow-dwelling animals also release hydrogen sulfide and produce sulfidic rich environments at the ocean floor.

The rRNA sequence based phylogenetic tree places a large number of thermophilic sulfur-respiring organisms near its root in Archea (71,81). Eukaryotes are most closely related to these Archaebacteria. In Eukaryotic genomes, there are certain sequences that are of disparate evolutionary origins and these are likely derived from Archaebacterial and Eubacterial cells (82-83). For example, in Eukaryotic cells, a specific vacuolar H^+-ATPase provides energy for the active transport across the vacuolar components of the endomembranes. In Eukaryotic cells, the amino acid sequences of the subunits of this enzyme are identical to the beta sub-unit of the Eubacterial-type F0F1-ATPases and 25% identical to their alpha subunits, suggesting an origin from an ancestral Archaebacterial-like cell. These sequences are also fully to 50% identical, respectively, to the alpha and beta subunits, of the sulfur-metabolizing *Sulfolobus acidocaldarius*, which is an Archaebacterium (84). Based on the phylogenetic tree of the subunits, it is thought that the gene duplication occurred to contain an Eocyte branch that gave rise to *Sulfolobus* and another Eubacterial branch that gave rise to Eukaryotic cells (84).

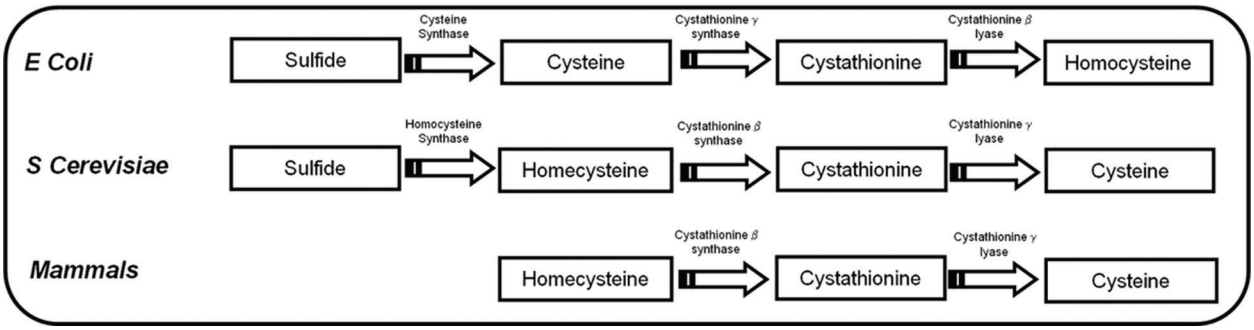

Figure 6. Metabolism of sulfides in different species.

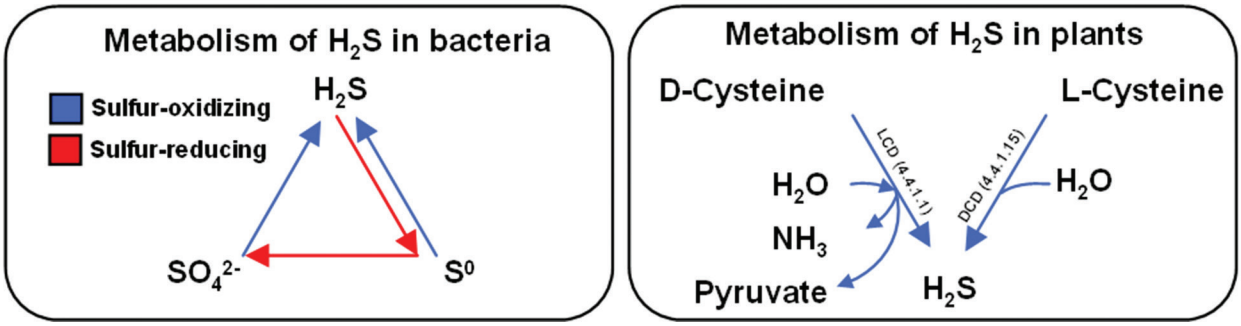

Figure 7. Metabolism of hydrogen sulfide in bacteria and plants.

The atmosphere of Earth has been modified several times by the need for generation of energy by organisms that inhabited Earth. During evolution, some microorganisms generated hydrogen sulfide, while plants contributed to oxygen and animals contributed to CO_2. Decomposition of plant proteins, by the action of a wide variety of actinomycetes, fungi, and bacteria, also releases hydrogen sulfide into the environment (85). In turn, nature uses hydrogen sulfide to change thiol metabolism in plants. For example, it has been shown that a short exposure to hydrogen sulfide is sufficient to increase the content of cysteine by 20-fold and glutathione by 4-fold in *Arabidopsis thaliana* (79).

During the early Archean (3.4 to 2.8 Ga ago), atmospheric oxygen was low and seawater had a low concentration of sulfate. The oxygen and sulfate began to accumulate in seawater in the early Proterozoic (2.5 to 0.54 Ga ago). Persistently high atmospheric levels of hydrogen sulfide during the Proterozoic is considered to have disrupted evolution of Eukaryotic life on Earth (73). It has been proposed that warming of the atmosphere led to an imbalance in growth of photosynthesizing plankton and sulfate-reducing bacteria in deep oceanic water. These bacteria generated massive amounts of hydrogen sulfide which made the oceans in-hospitable to most life forms. The release of hydrogen sulfide from oceans to the atmosphere, severely compromised the action of the ozone layer and caused mass extinctions by exposing the remaining life forms to fatal levels of UV radiation (86). Within a span of just 600 million years, the evolution of complex life forms was disrupted, at least by five mass extinctions. Dramatic increase in the level of hydrogen sulfide in the atmosphere or within the oceans is thought to have led, at least, to the Permian-Triassic extinction which occurred about 200-250 million years ago and likely to other extinction events (73). By the end of the Permian period, 95% of marine and 70% of terrestrial species became extinct. It is also thought that the enrichment of water with hydrogen sulfide, might have been responsible for the slowed recovery of marine ecosystems during the Early Jurassic (87). Despite the dramatic decrease in life forms when level of hydrogen sulfide increased in many different environments on Earth, life forms that survived and adapted for survival in hydrogen sulfide rich environments. Thus, the mass extinctions, guaranteed that a large number of organisms that remained were hydrogen sulfide dependent or tolerant, likely due to their reliance on hydrogen sulfide as their fuel or because of their tolerance due to their own production of hydrogen sulfide from organic matter. Hydrogen sulfide also further impacted evolution by forcing development of biodiversity. For example, the hotspots of Mesozoic biodiversity are shallow epicontinental seas which have been enriched several times by hydrogen sulfide.

The reliance on hydrogen sulfide for survival and protection against harsh environmental conditions

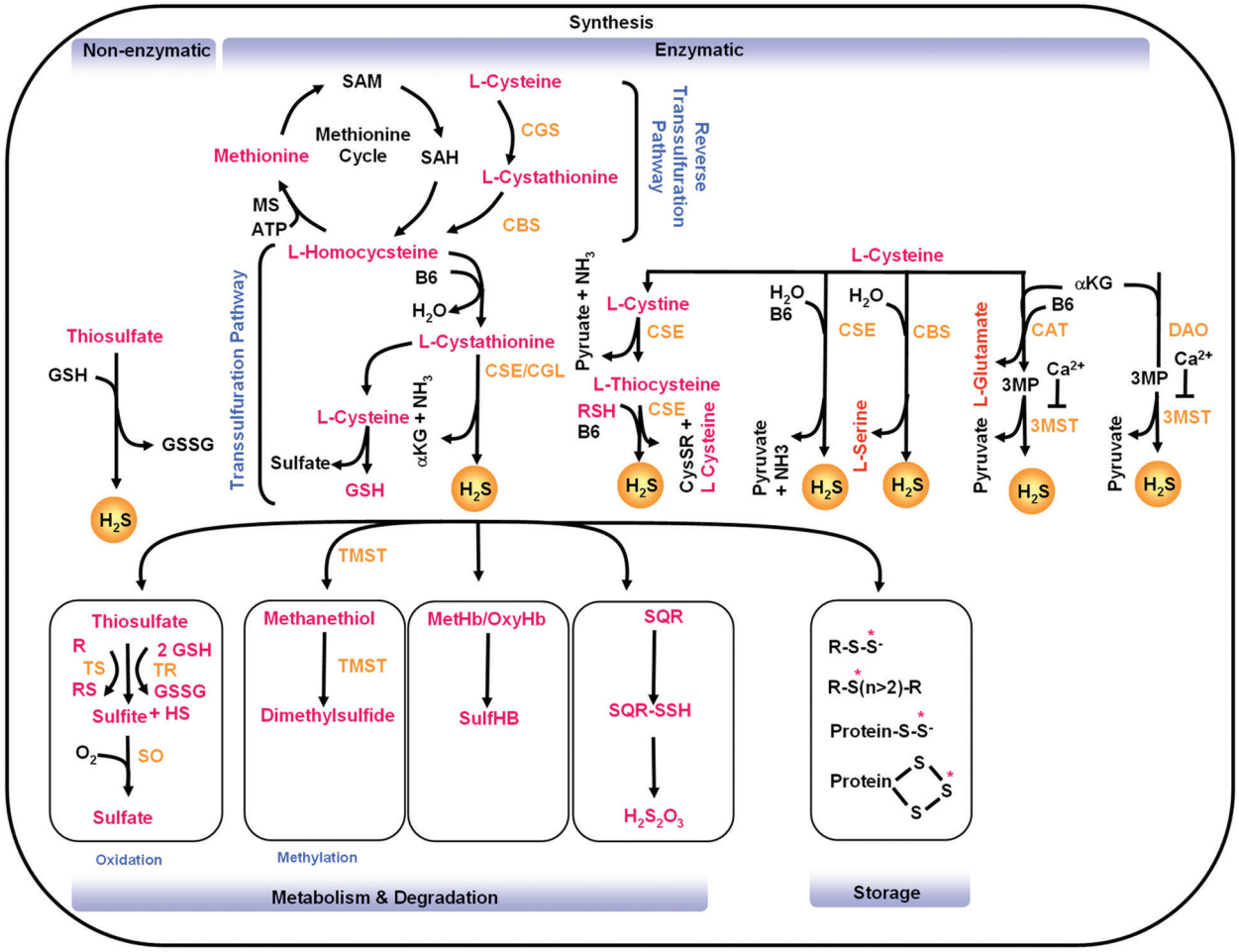

Figure 8. Pathways to synthesis, storage, release and degradation of hydrogen sulfide.

and utilization of hydrogen sulfide as a source of fuel has been preserved throughout the evolution. In modern cells, its synthesis and usage have become integrated into diverse signaling pathways. Because of such an importance, the synthesis of hydrogen sulfide is insured by various enzymatic and non-enzymatic reactions (Figure 8).The idea that animal cells are also capable to produce hydrogen sulfide, which was first reported in early 18th century by de Rey-Pailhade, is now well established (88). To generate hydrogen sulfide, animals and humans rely on receiving the dietary requirement of the amino acids such as cysteine and methionine that contain sulfur (Figure 8). Mammalian cells also actively generate hydrogen sulfide primarily by enzymatic and to a lesser extent by non-enzymatic reactions. In mammals, cysteine is synthesized from methionine by cystathionine beta synthase (CBS; EC 4.2.1.22), and cystathionine gamma lyase (CSE; EC 4.4.1.1) through the reverse trans-sulfuration pathway (RTSP) which also serves as a catabolic pathway for methionine and its toxic intermediates including homocysteine (Figure 8). Cysteine, in turn, is used for the biosynthesis of other organosulfurs such as glutathione and hydrogen sulfide. In mammalian cells, H_2S is synthesized primarily by the trans-sulfuration (TSP) and cysteine de-sulfuration pathways and through the de-sulfuration of cystine/cysteine by three enzymes; CBS, CSE and mercaptopyruvate sulfurtransferase (MST; EC 2.8.1.2). CBS and CSE are mainly produced in the cytosol while MST is found both in the mitochondria and cytosol (89). Animal cells also have a great capacity to increase their hydrogen sulfide production. For example, within seconds after intravenous administration of colloidal sulfur, animal cells produce substantial amount of hydrogen sulfide that is released from the lung and can be detected in the breath (90). In erythrocytes, glucose and all electron carriers including NADH, NADPH, and GSH stimulate production of hydrogen sulfide in cellular lysates (91). By reduction of elemental sulfur or inorganic polysulfides using reducing equivalents obtained from the oxidation of glucose, hydrogen sulfide is also non-enzymatically synthesized in small amounts under normal conditions but to a greater extent during oxidative stress or hyperglycemia (92) (Figure 8).

The use of hydrogen sulfide as fuel has remained intact throughout the evolution. For example, there is recent evidence that the majority of mammalian cells can avidly consume sulfides as a fuel by using the mitochondrial membrane flavoprotein, sulfide quinone reductase (SQR) (93). SQR oxidizes hyrogen sulfide to protein-bound persulfide. Sulfur dioxygenase and rhodanese, a sulfur transferase, respectively convert SQR-bound persulfide into sulfite and thiosulfate. Low molecular weight thiols such as glutathione or dihydrolipoate might also be involved in transferring the SQR-bound persulfide to the sulfur dioxygenase. Together, the available information shows that the use of hydrogen sulfide is intimately linked to the generation of energy in cells. Hyrogen sulfide catabolism also appears to be tied in with the electron transfer chain since ubiquinone, the electron acceptor of the electron transfer chain, transfers electrons to complex III.

4. DIVERSIFICATION OF SPECIES AND CELL FUNCTION REQUIRES REVISION OF METABOLIC PATHWAY

All modern cells generate energy by catabolism of organic compounds and energy is saved by conversion of adenosine 5′-diphosphate (ADP) to adenosine 5′-triphosphate (ATP). The energy stored in ATP is released by its conversion to ADP. The evolution and development of new cell functions drove changes in how energy was generated within cells starting from photosynthesis and glycolysis, to ultimately by oxidative metabolism. Primitive organisms, likely, extracted energy from their environment, initially by anoxygenic photosynthesis using chemicals, including sulfide, as electron donors. This was followed by anaerobic respiration using sulfate as an electron acceptor (resulting in the production of sulfide), and, finally aerobic respiration allowed organisms to use oxygen for generation of ATP by oxidative phosphorylation which significantly heightened energy generation and boosted the speed of evolution. In turn, the development of these metabolic pathways led to changes in the Earth atmosphere, and altered the course of evolution.

The evolutionary origins for the central glycolytic and pentose phosphate metabolic pathways in modern cells that create the essential precursors required for generation of amino acids, lipids and nucleic acids are poorly understood. However, based on the composition of early sediments, evidence was recently presented that similar to these pathways, the interconversion of metabolites occurred in oceans of the prebiotic Archean. These were comprised of 29 reactions that led to the formation and/or interconversion of glucose, pyruvate, the nucleic acid precursor, ribose-5-phosphate, and the amino acid precursor, erythrose-4-phosphate (42). Based on such an evidence, Keller *et al*, concluded that the origin of metabolism dates back to the prebiotic world. The evolution of the metabolic pathways, appears to be dependent on hydrogen sulfide. For example, it is hypothesized that the development of the self-organization of reductive citric acid cycle originated without enzymes or other informational molecules in an extremely reducing anaerobic environment that was enriched in hydrogen sulfide (94). In turn, these pathways enriched their environment through biogenic formation of hydrogen sulfide from sulfur and sulfate, processes which support acquisition of energy (94).

The absence of the conventional Embden-Meyerhof-Parnas (EMP) mode of glycolysis and common usages of modified non-phosphorylated Entner-Doudoroff (ED) pathways in *Saccharolytic Archaea* indicate that the ED pathway was an older pathway for dissimilation of carbohydrates. It is suggested that the EMP pathway was initially entirely anabolic and its catabolic role appeared later in evolution (95). The breakdown of glucose to pyruvate and then to lactic acid by anaerobic glycolytic pathway in modern cells is similar to the breakdown of organic molecules in the early life forms that lived in the anaerobic atmosphere of the early Earth. Glycolysis in these primitive organisms generated energy from breakdown of simple carbohydrates and released this energy which was stored in high-energy phosphate bonds.

Glycolysis is one of the ancient and most common metabolic pathways that is used, at least in part, by many organisms including human cells. In modern cells, it is used as the principal supplier of energy under hypoxic or other poor environmental conditions and when mitochondrial electron transport chain ceases to function (96). The generation of energy by this pathway is catalyzed by up to 12 enzymes in mammalian cells that, are exquisitely well characterized, in terms of their enzymatic activity as well as detailed structure including crystal structure (Figure 9). Glycolytic enzymes are amongst the most ancient and highly conserved genes and proteins across evolutionarily distant species from Prokaryotes to mammals (97). Comparisons of protein and gene structures of different species have demonstrated that throughout the evolution, genes have changed through insertions, deletions, exon-shuffling, duplication, fusion and lateral transfers. Similar changes are also seen during evolution, in the 12 enzymes that catalyze the metabolic reactions of the glycolytic pathway. Sequence alignment and comparison of glycolytic enzymes and a matrix of rRNA sequences have shown phylogenetic interrelationship in evolutionarily distant organisms such as Prokaryote, Archea, and Eucaryote superkingdom or ‘domain’, (98). For example, 3-phosphoglycerate kinases (PGK) of methanogenic Archaea are 30-60% identical to those in the Prokaryotes and Eukaryotes suggesting that all PGKs emerged from a common ancestor (98). The sequences of pyruvate kinase and lactate dehydrogenase also show remarkable similarity in species as diverse

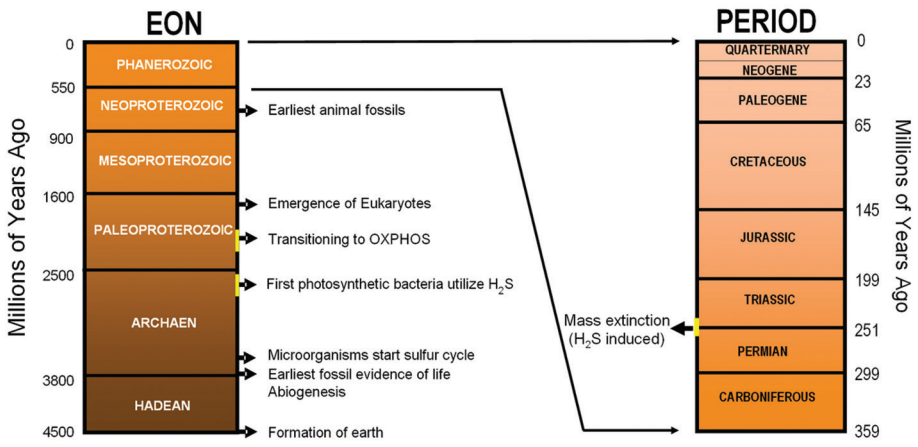

Figure 9. Milestones in evolution.

as yeasts to humans (97). It is also shown that the enzymes of this pathway have similar three-dimensional structures that have been strongly conserved throughout the evolution (99). Since there are four glycolytic kinases that catalyze similar reactions, one possibility is that throughout the evolution, an ancestral kinase may have led to the formation of other kinases. The strong conservation of enzymes of glycolysis shows that, during evolution, glycolysis metabolized similar substrates and effector molecules including nucleotides. However, because the pyruvate kinase and enolase are the only two enzymes of the pathway that are ubiquitous, it has been argued that this pathway might have evolved from the bottom up, allowing other enzymes to sequentially join the pathway during evolution (98).

The atmosphere of early Earth was poor in oxygen and was dominated by a highly reduced mixture of CH_4, CO, H_2S, and NH_3. However, this atmospheric condition changed around 2.4 Ga ago by what is known as the "Great Oxidation Event". The earliest species that survived the oxygen explosion during the 'Vendian' archeological period included Eukaryotic organisms such as sponges, hydra, filamentous algae and fungi (95). The switch from an oxygen-poor to an oxygen-rich Earth marks rapid changes in initiation of multi-cellularity, specification and differentiation of cells, generation of biodiversity and loss of immortality (Figure 9). Based on understanding as what is required for life to form, detection of gaseous biosignature of "oxic" biospheres is considered to include (O_2), and ozone (O_3), in presence of reduced species such as methane (CH_4). However, since life was present well before significant O_2 accumulated in the atmosphere, sulfur gases are used for remote detection of biosignatures of anoxic planets (100).

During the last two billion years, the generation of energy and its regulation became oxygen dependent in many species (Figure 9). The variations in oxygen tensions led to generation of systems that coordinately and simultaneously regulated multiple un-linked genes of glycolysis and oxidative phosphorylation throughout the genome and mitochondrial DNA. Oxidative phosphorylation is the principal supplier of energy in Eukaryotic cells, at normal pO_2 levels. However, when the pO_2 falls below a critical level, mitochondrial electron transport chain ceases to function and anaerobic glycolysis gets activated. In cancer cells, however, glycolysis is activated even in presence of ample oxygen, a phenomenon which was identified by Warburg and is known as Warburg's effect or aerobic glycolysis (100). Glycolysis is the more ancient pathway that is maintained throughout the evolution to be used as the principal supplier of energy under hypoxic or other poor environmental conditions and when mitochondrial electron transport chain stops to function (96) (Figure 9). Damage recovered cancer cells opt this more archaeic pathway for generation of energy, damage recovery, for induction of damage tolerance, and for adopting a primitive cell type, phenotypes that these cells have in common with stem cells (Figure 10) (96,102-105).

5. EVOLUTION OF METABOLISM LEADS TO BIODIVERSITY AND DIVERSIFICATION OF CELL FUNCTION THROUGH ENDOSYMBIOSIS

In energy production, glycolysis is quite in-efficient, with each cycle giving rise only to 2 ATP molecules. Moreover, glycolysis is dependent on release of energy from organic molecules which is stored in ATP used in all energy dependent reactions. In the next major presumed evolutionary step, this reliance of release of energy from organic molecules was lifted with the development of photosynthesis (Figure 9). This new pathway allowed cells to directly harness energy from sunlight. One possibility is that the abundance of microorganisms that utilized photosynthesis led to the release of O_2 and development of an oxygen

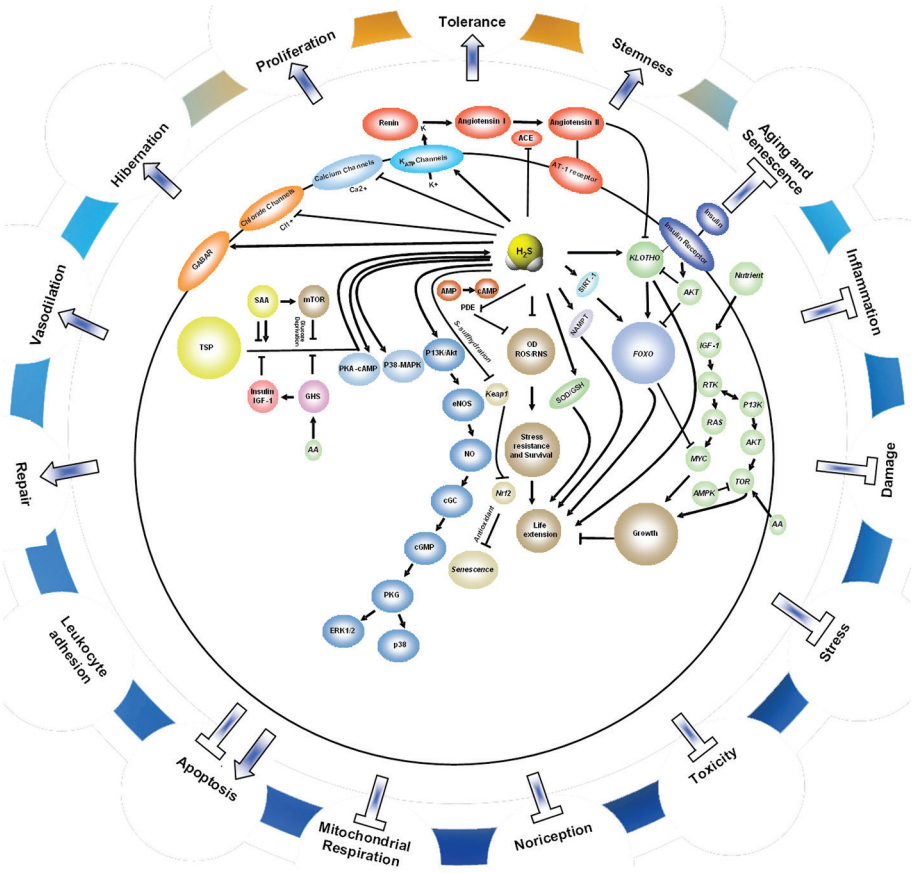

Figure 10. Pathways impacted by hydrogen sulfide and their impact at organismal level.

rich atmosphere. Such an atmosphere might have led to the development of oxidative phosphorylation within an ancestral microorganism that now exists as an endosymbioant in the present day cells. Alternatively, oxidative metabolism might have preceded photosynthesis so that the increase in atmospheric O_2 provided a selective advantage to microorganisms that utilized oxygen for generation of energy. It is estimated that mitochondria might have become endosymbioant close to 2 Ga or earlier after prokaryotic cells arose which then allowed the citric acid cycle and oxidative phosphorylation to develop.

According to the endosymbiotic or symbiogenesis hypothesis, life did not populate Earth just through competition but also through collaboration, networking and adaptation. According to this hypothesis, around 1.5-2.3 Ga ago, mitochondria and several other organelles in Eukaryotes were once free living single-celled bacteria. Mitochondria and chloroplasts are similar to bacteria in terms of their size, and contain their own DNA, and like cells, they synthesize proteins and can replicate. The similarity of mitochondrial DNA genome to bacterial genomes and other molecular and biochemical evidence have suggested that mitochondria have evolved from aerobic bacteria, which are the Eubacterial ancestors of a subgroup of the α-Proteobacteria (106). Similarly, the chloroplasts are thought to have originated from endosymbiosis in cyanobacteria (107-108). Subsequent to the endosymbiosis, over time, many bacterial genes such as ~1500 genes of the mitochondrial genome were transferred by endosymbiotic gene transfer to nuclear DNA and these genes are now scattered throughout the chromosomal DNA. Today, the maternally inherited mitochondrial DNA retains only the genes which are required for mitochondrial protein synthesis including the 12S and 16S rRNAs, the 22 tRNAs, and 13 polypeptides that participative in diversity of mitochondrial functions including oxidative phosphorylation (OXPHOS) and energy generation (109).

The evolution of microorganisms was initially slow, likely due to their in-efficient energy production. After adoption of highly efficient oxidative phosphorylation, with each Krebs cycle being able to generate around 36 ATP molecules, evolution speeded up so that all current species developed only in a span of approximately 2 billion years (110). The mitochondrially derived energy boost provided the opportunity for rapid and significant increase in pace of evolution allowing single cells to

initially colonize. Colonization, which is still seen in some modern organisms such as algae, might have served as an intermediary step in the evolutionary transition of single cells into multicellular organisms. From almost 1.7 Ga ago, the oxidative phosphorylation fostered evolution, led to formation of multi-cellular organisms, generated diversity in cell specialization, allowed division of labor and opened the path for the development of all present-day species.

The use of hydrogen suflide in endosymbioants, living in oxygen rich Earth, continued and throughout the evolution, a reciprocal relationship developed between oxygen and hydrogen sulfide. Hydrogen sulfide is a universal and phylogenetically ancient oxygen sensor, being integrated to the regulation of oxygen available to cells as well as into the machineries that protect cells that face hypoxic conditions (111-112). Such interaction includes hydrogen sulfide production and/or metabolism being biochemically coupled to oxygen so that the tissue concentration of hydrogen sulfide is in tune with the pO_2. Throughout the evolution, cells first evolved to deal with a sulfidic and anoxic (euxinic) Earth and then subsequently were exposed slowly to an increasing level of oxidants; namely, oxygen. As a result of such adaptations, mitochondria in modern cells use hydrogen sulfide as an oxygen sensor, as a source of fuel and in mitochondrial signaling (111-112). Several lines of evidence support the view that hydrgen sulfide and hypoxia act via common effector pathways. The cellular production of hydrogen sulfide is inversely related to physiologically relevant levels of pO_2. Moreover, concentration of biologically active hydrogen sulfide, is tightly regulated by its constitutive production as well as by its oxidation in mitochondria. In turn, the mitochondrial respiration is controlled by hydrogen sulfide which blocks the respiratory chain primarily by inhibiting cytochrome c oxidase (112-113). Exogenous H_2S production by its agonists produces a physiological response which is similar to hypoxia and H_2S simultaneously protects cells against hypoxia and hypoxic damage. On the other hand, antagonists of biosynthesis of hydrogen sulfide exacerbate hypoxic responses. Together, such evidence show that hydrogen sulfide and oxygen are intricately involved in tightly regulating each other's balance and in the absence of adequate oxygen, hydrogen sulfide, provides protection against hypoxic damage (114-120).

6. ENDOSYMBIOSIS INDUCED POST-MITOTIC DIFFERENTIATION MARKS THE DOWN OF MORTALITY

Endosymbiosis gave a rapid pace to evolution but it also brought mortality to the new hybrid cells. In many multi-cellular organisms, these hybrid cells diverged and generated two sets of cells; the un-differentiated stem cells with cell renewal property similar to the primitive cells on Earth and the differentiated cells that lose such an ability but, in turn, produce highly evolved post-mitotically differentiated cells that are mortal and are subject to aging.

It is thought that the cause of aging might be attributed to the sequential accumulation of un-repaired damages that lead to a gradual decline in structure and function of the cell. Many theories have been proposed for development of aging, including the free-radical, mitochondrial and stem cell theories of aging. Originally proposed by Denham Harman, the free radical theory proposes that aging results from sequential accumulation of damage by free oxygen radical species (ROS) (121). ROS are mostly generated at the electron transport chain as the end by-products of mitochondrial aerobic respiration. To a lesser extent, ROS are also produced by other catabolic and anabolic cell processes (122). ROS are comprised of a number of highly reactive chemical species including superoxide anion (O^{2-}), hydroxyl radical ($^{\cdot}OH$), and hydrogen peroxide (H_2O_2). These species cause oxidative damage to protein, lipid and nucleic acids including generation of mutations, oxidized DNA bases 7,8-dihydro-8-oxo-deoxyguanosine (8-oxo-dG), abasic sites, and DNA strand breaks. According to the stem cell theory, aging results from accumulation of damage in stem cell compartments. Consistent with this theory, genetic studies of mice deficient in genes implicated in ROS regulation indicate that elevated level of ROS within the stem cell compartments leads to a rapid decline in stem cell self-renewal (123-126). A high level of ROS decreases the ability of long-term self-renewal in hematopoietic stem cells (HSC) and treatment of these cells with the antioxidant, N-acetyl cysteine, restores the functional activity of these cells (127). Taken together, these studies provide a persuasive argument that ROS play an important role in aging.

The mitochondrial theory of aging, extended from the free radical theory, proposes that oxidative damage of mitochondrial macromolecules such as mitochondrial DNA, proteins, or lipids is responsible for aging and that all conditions that harm mitochondria or generate mitochondrial genetic defects lead to aging associated degenerative diseases such as diabetes, neurodegenerative diseases and cancer. Studies in mutant and transgenic mice have shown that mitochondrial reactive oxygen species (ROS) limit the life-span of mammals (128). Thus, it is postulated that the daily production of ROS by mitochondria leads to a gradual deterioration of structure and decline in function of the mitochondria and leads to failure to repair or to rebuild the damaged structures and to restore functions. It has been shown that oxidative damage perturbs replication and transcription of mitochondrial DNA and leads to a decline in mitochondrial function (129). Mice expressing proof reading-deficient mitochondrial DNA polymerase, which consistently showed increase in mitochondrial DNA mutations, underwent premature

aging (130-131). Mutations in mitochondrial DNA by oxidative damage impairs either the assembly or the function of the respiratory chain, which in turn, leads to further accumulation of ROS, energy depletion and ultimately causes cell death (121,128,131-132). Oxidatively damaged mitochondria are thought to lead to aging through inducing apoptosis in cells such as those that have endured oxidative modification of mitochondrial adenine nucleotide translocase, a component of the permeability transition pore (133). Besides mitochondrial DNA mutations and deletions, aging might also be related to the decline in the abundance of mitochondrial DNA which correlates with the rate of mitochondrial ATP production (134-135).

Hydras are freshwater organisms that possess mitochondria, however, they are exceptional since they do not undergo senescence, and, for this reason they are biologically immortal (136). The immortality of these organisms might be attributable to the fact that Hydras do not have post-mitotically differentiated cells, rather, their cells constantly divide. For this reason, in contrast to most organisms that have post-mitotic differentiated cells, the cells in Hydras do not accumulate damage. Thus, it can be reasoned that because diversification of cell function and differentiation became feasible only in presence of mitochondria, conditions that reduce reliance on mitochondria and promote glycolysis as the primary pathway of metabolism should resume primitive cell routines, a feature that stem cells and cancer stem cells have in common.

7. HYDROGEN SULFIDE SUSTAINS LIFE

The impact of hydrogen sulfide in sustaining life has been conserved throughout evolution. Hydrogen sulfide is essential to life, it prolongs life-span, reduces the impact of age related diseases, protects life against damage and harsh environmental conditions and promotes and sustains stemness.

8. HYDROGEN SULFIDE EXTENDS LIFE-SPAN

Hydrogen sulfide has life promoting effect. In plants, hydrogen sulfide improves root organogenesis and seed germination (137). In mice, homozygous knockout (KO) of CBS led to a reduced lifespan to around 4 weeks (138). CSE-KO mice also show growth retardation and exhibit a shortened life span on a low cysteine diet indicating that, in the absence of CSE, sufficient cysteine supply is a prerequisite for animal survival (139).

Hydrogen sulfide significantly extended the life-span in *Caenorhabditis elegans* and slowed down the aging by reducing oxidative stress through its anti-oxidant activity, and by inhibiting free-radical reactions by regulation and its interactions with *SIRT1* (140) (Figure 10). The silent information regulator 2 (*SIR2*) gene family is conserved from Prokaryotes to Eukaryotes (*Staphylococcus aureus*, fission yeast, *Arabidopsis*, *Caenorhabditis elegans*, mouse, rat, human). The members of this family which silence certain cell functions, are involved in cell cycle progression, and provide chromosomal stability (141). The life-extension function of *SIRT1* might be related to its ability to deacetylate the *FOXO* transcription factors which promote resistance to cellular stress (142). Mammals have seven *SIR2* homologues (SIRT1-7). Among these, *SIRT1* is most closely related to *SIR2*. Sir2 proteins are good candidates as regulators of life span since these proteins have NAD-dependent sirtuin core domain, which has remained highly conserved in many different species (143). *SIR2* has been shown to be a determinant of life span in yeast mother cells. *SIR2* induces longevity in *Saccharomyces cerevisiae* by suppressing the generation and accumulation of extrachromosomal rDNA circles, which is considered to cause aging in yeast (144). *SIR2* also encodes a histone deacetylase, which is reported to extend the lifespan of *Caenorhabditis elegans* through signaling pathways that involve *DAF-2/INSULIN Receptor* and *DAF-16/FOXO* (145-146) (Figure 10).

The life extending property of hydrogen sulfide is also attributed to the regulation of *KLOTHO* which suppresses the expression of multiple age-associated phenotypes. *KLOTHO* represses *DAF-2* (Insulin/IGF-like) receptors by inducing de-repression of *DAF-16* (*FOXO*) and activates the *FOXO* forkhead transcription factors. These factors, in turn, induce expression of manganese SOD which increases resistance to oxidative stress, produces stress resistance and improves longevity (147-148) (Figure 10). Also it is possible that the life promoting activity of hydrogen sulfide might be through increasing the activity of endogenous inhibitor of phosphodiesterase since the anti-aging effect of resveratrol, that prevents age related metabolic damage of aging, has been attributed to inhibition of cAMP phosphodiesterases (149-150). Embryonic fibroblasts isolated from CSE knockout mice display increased oxidative stress and accelerated cellular senescence. Incubation of cells with NaHS, a H_2S donor, significantly increased the level of glutathione and rescued these CSE deficient cells from senescence. Protection against cellular senescence by hydrogen sulfide has been attributed to S-sulfhydration of Keap1 which acts as a negative regulator of Nrf2, a master regulator of the antioxidant response (151).

There is also the possibility that hydrogen sulfide might have other life-extension properties such as the potential to activate transcription machineries that prevent damage, and promote repair. Hydrogen sulfide prevents the damages that lead to aging. Most biological macromolecules contain nucleophilic centers and their electrophilic byproducts such as nitrated cyclic nucleotide, 8-nitroguanosine 3′,5′-cyclic monophosphate

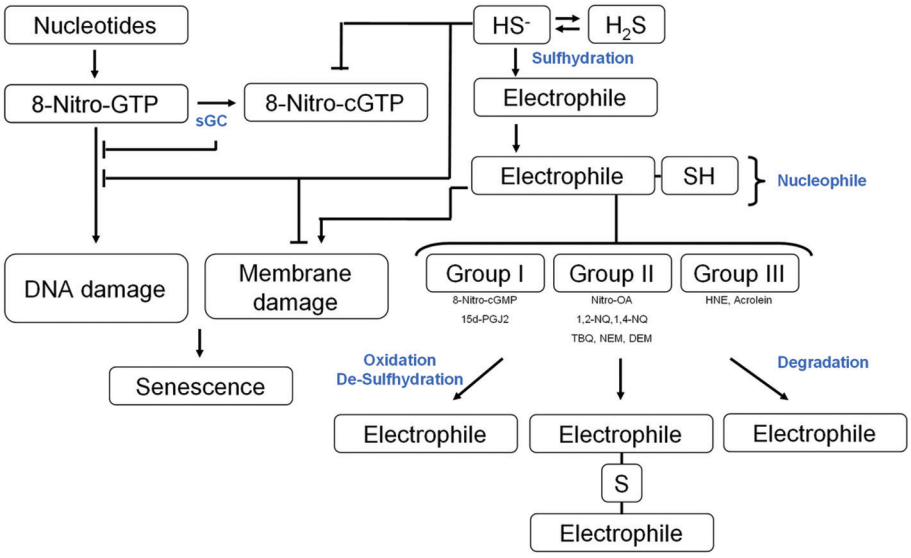

Figure 11. Impact of hydrogen sulfide and HS^- on metabolism of electrophiles and prevention of DNA damage and senescence.

(8-nitro-cGMP) and nitro or keto derivatives of unsaturated fatty acids are important to redox signaling (152). These reactive compounds are generated by reactions of reactive oxygen species, nitric oxide and secondary products as well as enzymes released during inflammation. Majority of electrophiles are derived by oxygen-centered radicals from polyunsaturated fatty acids by a peroxidation chain reaction. Most oxidized biological macromolecules are particularly reactive and toxic to biologic mechanisms and life span appears to be inversely linked to the peroxidation events on the cell membrane (153). It is thought that the formation of lipid-derived electrophiles such as 4-hydroxynon-2-enal (4-HNE) provides a link between high susceptibility of membrane lipids to peroxidation and shortened life span since it has been shown that the direct alteration of membrane composition (and thus their peroxidizability) or modulation of 4-HNE levels impact life span, providing a causal role for such compounds. It has been shown that 4-HNE can destabilize biological systems and modulate signaling pathways that control longevity (154). The beneficial impact of increased hydrogen sulfide might be by virtue of modulation of redox signaling. Redox signaling is the pathway mediated by electrophilic byproducts. It is now apparent that hydrogen sulfide anion (HS^-) regulates the metabolism and signaling actions of various electrophiles (Figure 11). For example, HS^-, suppresses electrophile-mediated H-Ras activation and cell senescence in cardiac cells (152). Hydrogen sulfide deactivates three group of electrophiles by enzymatic release of HS^- group. The SH derivative of group are stable, however, they can be degraded by oxidation and removal of SH group by ROS. The product of second group form relatively stable bis products (bis-S-compounds). The third group of electrophiles show extensive HS-induced degradation, likely by specific sulfhydration. 8-nitro-cGMP is formed from its precursor, 8-nitro-GTP, by catalytic action of sGC. HS^- reacts with electrophiles, such as 8-nitro-cGMP, via direct sulfhydration and modulates cellular redox signaling (152). GTP in the nucleotide pool of cells is nitrated to 8-Nitro-GTP and its accumulation is thought to lead to increased DNA damage and mutagenesis and hence to a halt in repair mechanisms and sensensce (152). Together, the available evidence is consistent with a role for hydrogen sulfide in providing protection against nuelophilic interactions that shorten lifespan.

9. THE LIFE EXTENSION PROPERTY OF CALORIE RESTRICTION DIET IS MEDIATED BY HYDROGEN SULFIDE

The term “hormesis” was coined in 1943 by Southam and Ehrlich as enhanced survival as a result of exposure to low-intensity biological stressors, such as calorie restriction, hypoxia/ischemia, endogenous oxidant stressors, heat, radiation, exercise, and toxic compounds (155). Some consider that the age promoting effect of such an stress might be directed at mitochondria where, a coordinated response to mild mitochondrial stresses, make the cells resistant to future stresses (mito-hormesis) (156). Such a damage resistant state is thought to be counteracted with vitamin C, vitamin E as well as by sulfur containing amino acid, cysteine while reduction of dietary methionine and cysteine increases longevity (157-162).

Dietary or calorie restriction (DR or CR) is a dietary regimen that reduces the calorie intake without imposing malnutrition. In a number of species, such as yeast, *Saccharomyces cerevisiae*, *Drosophila melanogaster*, worms, *Caenorhabditis elegans,* flies, fish, rodents, dogs, and perhaps non-human primates,

this diet has been shown to increase longevity, and to provide stress resistance (163-164). A number of studies in various model organisms suggest that the increase of lifespan can be achieved by reducing oxidative stress and reactive oxygen species (ROS) (157). However, many prospective clinical trials have so far failed to show any beneficial effect of anti-oxidants and some even have suggested that the increased formation of (ROS) within the mitochondria induces an adaptive response that increases stress resistance causing a long-term reduction of oxidative stress (157). Amino acids, in general, appear to promote aging by increase in mTOR pathway, while their deficiency, particularly of methionine leads to life extension by reducing IGF-1 signaling (161,165) (Figure 10). While restriction of methionine delays aging in rodents, presence of methionine in the diet has been shown in *Drosophila melanogaster* to counteract the lifespan extension effect of CR (161,164).

The extension of life-span by hydrogen sulfide has been shown in *Caenorhabditis elegans* (166-167). However, such a life extension was considered to be distinct from that afforded by calorie restriction or by inhibiting insulin signaling or mitochondrial function (166-167). Recently, Hine *et al* have proposed that the life extension by calorie restriction is mediated through hydrogen sulfide (158-159). Hine *et al* show that both CR and hydrogen sulfide extend life span in flies, yeast and worms (158-159). In worms, the knockdown of CBS, a major enzyme that leads to hydrogen sulfide production decreased *eat-2* mediated life-span extension while its over-expression led to an increase in life span (158-159). Restriction of calorie, and of amino acids, methionine and cysteine, were all sufficient to cause a significant increase in the hydrogen sulfide which could be blocked by activation of mTORC1 suggesting that this pathway works either downstream from the IGF-1 signaling or that these amino acids can directly inhibit hydrogen sulfide production by activating this kinase. One possibility is that, in face of calorie or protein deficiency, hydrogen sulfide donates electrons by virtue of the mitochondrial enzyme sulfide quinone oxidoreductave (SQR) to the mitochondrial proteins such as co-enzyme Q, which has been shown to protect cells against damage. Thus, hydrogen sulfide appears to act at the mitochondrial level to reduce oxidative stress, and to protect cells in case of protein deficiency.

It was reported that calorie restriction increases Sir2 activity and activates Sir2 deacetylase in yeast and *Drosophila*. Since *SIR2* homologues are NAD^+-dependent deacetylase enzymes that may have a variety of substrates, one possibility is that hydrogen sulfide shifts redox homeostasis, by increasing NAD^+ or by changing the NAD^+/NADH ratio, thus resulting in the increased activity of *SIR2.1 (*102,167). Another possibility is that hydrogen sulfide directly induces *SIRT1* activity since in human umbilical vein endothelial cells, the inhibition of aging by hydrogen sulfide was reported to be through regulation of *SIRT1* activity (168-169) (Figure 10).

10. HYDROGEN SULFIDE PROTECTS LIFE AGAINST AGE RELATED DISEASES

The production of hydrogen sulfide decreases with age. For example in *Arabidopsis,* the levels of mRNA of L-cysteine desulfhydrase (L-CD) and D-cysteine desulfhydrase (D-CD) are gradually elevated in a developmental and stage-dependent manner, with significantly higher levels present in juvenile compartments of the plant such as stems, cauline leaves and flowers (170). The concentration of hydrogen sulfide in the leaves of *Arabidopsis thaliana* varies from 1 to 15 μM with much higher concentrations in the younger rather than older leaves or older plants (171). In humans, the plasma concentration of hydrogen sulfide also decreases with age and its concentration is even more decreased in age related diseases such as Alzheimer's disease, hypertension, diabetes and atherosclerosis (172-174). This reduced level of hydrogen sulfide might contribute to the severity of such age-related diseases. As compared to levels in normotensive control animals, the concentration of hydrogen sulfide in plasma was markedly lower in hypertensive rats (174-175). The vasodepressive effects of hydrogen sulfide have been attributed to induction of hyperpolarization of smooth muscle cells that causes vaso-relaxation through effects mediated by ATP-sensitive K^+ (K_{ATP}) channels. By cysteine S-sulfhydration, hydrogen sulfide, increases the activity of ATP-sensitive K^+ (K_{ATP}) channels by enhancement of binding of Kir6.1-PIP2 while reducing the binding of Kir6.1-ATP (176) (Figure 10). Increases in intracellular calcium and activated calcium-dependent calmodulin by stimulation of muscarinic cholinergic receptors on endothelium, activates CSE and generates hydrogen sulfide which acts on both endothelial cells and vascular SMCs to cause vasorelaxation. Consistent with this role, at the age of 8 weeks, the CSE-KO mice develop hypertension (177). Moreover, hydrogen sulfide reduces the proliferation of vascular smooth muscle cells which is a feature of hypertensive pulmonary vascular disease in humans (178). The disruption of homeostasis of hydrogen sulfide might also underlie the pathogenesis of atherosclerosis since it has been shown that hydrogen sulfide has anti-atherogenic properties by suppressing the formation of macrophage-derived foam cells (179-180).

Reduced levels of hydrogen sulfide appear to be contributing to the pathogenesis of several neurodegenerative diseases such as Parkinson's and Alzheimer's disease (181-182). In a model of Parkinson's disease, induced by 6-hydroxydopamine (6-OHDA), the level of hydrogen sulfide was markedly reduced in substantia nigra, which, in this disease, shows a progressive loss of dopaminergic neurons. Administration of hydrogen sulfide has neuroprotective effect, it inhibits several effects of 6-OHDA including the activation of NADPH

oxidase in substantia nigra and prevents accumulation of inflammatory mediators in the striatum (181,183). It has been shown that the brain of patients with Alzheimer's disease, has a reduced level of hydrogen sulfide and, in such brains, homocysteine accumulates to levels that cause neurotoxicity. This elevated level of homocysteine has been shown to be associated with down-regulation of expression and activity of CBS, the major hydrogen sulfide producing enzyme in the brain which leads to a decrease in the endogenous level of hydrogen sulfide (172,184). On the other hand, it has been shown that hydrogen sulfide can protect against homocysteine-induced neurotoxicity (184). In a model of amyloid beta induced cell injury, the neuroprotective effect of hydrogen sulfide has been shown to be through preservation of mitochondrial function in a p38- and JNK-MAPK-dependent manner, and by inhibiting infla mmation, and p`romoting cell growth (185).

The level of hydrogen sulfide has also been reported to be reduced both in type 2 diabetes and in streptozotocin-treated diabetic rats (173). Under normal conditions, hydrogen sulfide protects endothelial cells from hyperglycemia-induced endothelial dysfunction by attenuating the hyperglycemia-induced enhancement of cell viability by reduced formation of ROS and DNA injury (186). The level of hydrogen sulfide in plasma and aortic tissues are also progressively reduced in non-obese diabetic (NOD) mice (187). It has been proposed that this low level of hydrogen sulfide might be due to its active degradation caused by mitochondrial ROS overproduction (186).

11. HYDROGEN SULFIDE PROMOTES LIFE BY IMPOSING DAMAGE TOLERANCE

Hydrogen sulfide is intricately linked to many cell processes that protect plants to mammalian cells from damage (188-189). Sulfur is one of the critical macronutrients that impact crop yield by affecting growth and vigor of plants. Fertilization with sulfur induces the so-called sulfur-induced resistance (SIR) in plants against a variety of pathogens (170). Plants also constitutively or under stress synthesize a host of endogenous sulfur-containing defense compounds (SDCs) such as glucosinolates, phytoalexins, elemental sulfur, glutathione, phytochelatins, various secondary metabolites, and sulfur-rich proteins (190). In plants, hydrogen sulfide increases tolerance to freezing and drought (191-192). Drought significantly upregulates the expression of *L-CD* and *D-CD* in plants that increases production of hydrogen sulfide by six- to sevenfold. Exogenous hydrogen sulfide, in turn, increases the expression of drought-associated genes (*DREB2A*, *DREB2B*, *CBF4*, and *RD29A*) and leads to a significant increase in drought resistance (192).

In mammalian cells, exposure to hydrogen sulfide decreases metabolism and increases tolerance of cells to hypoxia, and hypoxic injury. For example, Mark Roth showed that mice that were exposed to hydrogen sulfide entered a hibernating state that was associated with decreased metabolism and, consequently, with reduced heart and respiration rates (193). Yet, when the hydrogen sulfide was withdrawn, mice did not show any evidence of neurologic damage despite the dramatic drop that they endured in terms of both heart or respiration rate (193). Hydrogen sulfide affords cytoprotection in hypoxia and ischemia–reperfusion model in various organs including liver, lung, kidney and particularly heart (114-120). Hydrogen sulfide protects cells against other types of injury including oxidative injury, endotoxic shock, and other types of cell damage and cell death (119,194-198) (Figure 10). Thus, in presence of hydrogen sulfide, cells gain a survival advantage to tolerate hypoxia and other harsh microenvironmental conditions that otherwise can lead to cell death.

Increased generation of H_2S plays an important role in dictating cell survival after severe damage by promoting a reduction-oxidation balance, suppressing oxidative stress in mitochondria and increasing glutathione production (199). Increase in the level of ATP and NAD^+ in cancer cells that recover from damage *in vitro* is due to up-regulation of H_2S production since forced increase in intracellular level of H_2S leads to a concomitant rise in cellular pool of ATP and NAD^+ (102-103). Cancer cells that recover from damage derived from tumors generated *in vivo* also exhibit a high level of H_2S and Nampt and concomitantly show increased glycolysis, ATP and NAD^+ production. Inhibition of cystathionine beta synthase (CBS) or cystathionase (CTH) enzymes, that drive hydrogen sulfide production in cancer cells, decreases Nampt production that drives ATP generation while suppression of Nampt pathway by FK866, a highly specific non-competitive inhibitor of Nampt, decreases H_2S and simultaneously decreases the cellular pool of ATP (103-104). Consistent with such data, it has been shown that FK866 which depletes the cellular pool of NAD^+, induces tumor cell apoptosis while nicotinic acid and nicotinamide oppose such effects of FK866 (200). In line with these observations, H_2S is shown to improve mitochondrial ATP production following hypoxia (201). FK866 causes attenuation of glycolysis at the glyceraldehyde 3-phosphate dehydrogenase step and, in turn, restricted carbon flow from glycolysis results in reduced serine biosynthesis (202). Therefore, FK866 mediated inhibition of H_2S production might be linked to reduced biosynthesis of serine and its utilization by CBS, which produces H_2S and cystathionine (Figure 10). Cystathionine, in turn, is utilized by CTH to further produce H_2S and cysteine. The existence of this positive feedback loop between H_2S and Nampt may contribute to glycolytic activity and survival of cancer cells in face of microenvironmental challenges as well as after drug treatment. According to Oncomine database, H_2S-producing enzymes, CBS, CTH and MST as well as Nampt

are all overexpressed in cancers of liver and breast and in melanomas (https://www.oncomine.org/) suggesting that this pathway operates in cancers of diverse origins.

The cyto-protective effects of H_2S is related to its ability to increase cellular pool of ATP and NAD^+ through a Nampt mediated response. Cancer cells, which are treated with H_2S donor, NaHS, and cancer cells which recover from damage show an increased CBS and CTH driven H_2S synthesis and both have increased Nampt, the rate-limiting factor in NAD^+ biosynthesis, and its product NAD^+. Such an increase should enable cells to resist death that is induced when supplies of ATP and NAD^+ are exhausted. Another possibility to consider is that, some, if not all of the increase in ATP in cancer cells, might be due to the direct utilization of H_2S as a substrate in ATP generation by cancer cells. Recent evidence suggests that, in mammalian cells, H_2S can serve as an electron donor and an inorganic source of energy. While low concentrations of H_2S (0.1-1 μM) elicit a significant increase in mitochondrial function, higher concentrations (3-30 μM) have an opposite effect on cellular bioenergetics (203). Based on such data, it has been suggested that intramitochondrial H_2S complements and balances the bioenergetic role of Krebs cycle-derived electron donors (203).

12. HYDROGEN SULFIDE INITIATES, PROMOTES AND MAINTAINS STEMNESS

Stem cells are un-differentiated cells that can give rise to all terminally differentiated cells in various tissues and organs of multi-cellular organisms. Cancer stem cells also are un-differentiated cells that maintain the pool of differentiated cancer cells. Additional similarity between cancer stem cells and stem cells becomes evident when their gene expression profiles are compared. A ‘‘core’’ Embryonic Stem Cell (ESC)-like gene module comprised of 335 genes, is expressed and activated in multiple human cancers but repressed in normal tissues (204). Of these, eighty-eight of the module genes are *c-MYC* targets indicating that modulation of few core genes might be sufficient to drive the expression of all the genes in this module. *OCT4*, *NANOG, CRIPTO or REX1* are part of a transcriptional network that activate genes that promote stem cell growth and self renewal while simultaneously repressing genes that promote differentiation (205-207). It is now recognized that the molecular signature of cancer stem cells is similar to that of embryonic stem cells (ESC). The expression of key factors that control stem cell identity such as *OCT4* has been reported in a variety of cancers including hepatocellular, gastric and cervical carcinomas (208-211). It has been suggested that $OCT4^+$ cancer cells might be tumor-initiating stem cells, a concept that is consistent with the role of this transcription factor in the maintenance of stem cell pluripotency. However, the presence of embryonic *OCT4A* in tumor tissue and tumor cell lines was doubted by some who attributed the observed *OCT4* expression to the expression of *OCT4* pseudogene (212-213). On the other hand, the expression of another pluripotency gene, *SOX2* (Sex-determining region Y (SRY)-Box2) has been reported in various cancers and cancer cell lines (Herreros-Villanueva M, *et al.* 2013; Dogan I, *et al.* 2014; Amini S, *et al* 2014). It has been suggested that *SOX2* contributes to the de-differentiation of cancer cells endowing them with highly aggressive properties (210,214). *OCT4* and *SOX2* cooperatively stimulate the transcription of several downstream target genes such as *NANOG*, *DPPA2* (developmental pluripotency-associated two), and *FBXO15*, which also show oncogenic transforming activity (215-217).

Whereas mitochondrial metabolism directs differentiation and inhibits stemness, glycolysis appears to be the preferred mode of metabolism in normal and cancer stem cells as well as damaged cells and senescent cells (218). Glycolysis maintains stemness or senescence, likely by minimizing generation of reactive oxygen species and halting lineage-specific differentiation (219,220). Stem cells use the glycolytic flux for satisfying their need for energy as well as for generation of bio-mass required for cell division. A distinctive feature of stem cells is their capacity for self-renewal and many types of stem cells rely heavily on anaerobic glycolysis (123,221-222). Modified mitochondrial function and promotion of aerobic glycolysis are key to the induction of pluripotency and its maintenance (223). It is becoming clear that mitochondrial and metabolic-related processes are intertwined with signaling and epigenetic rewiring, that direct stemness, cell fate decisions and tissue repair following damage as seen in senescent cells (224-225). Besides generation of NADPH, and ATP, one primary reason for re-wiring of metabolic pathways and adoption of glycolysis is participation of this pathway in cell survival, and cell growth by anabolic production of the intermediate macromolecular precursors such as acetyl-CoA for fatty acids, glycolytic intermediates for non-essential amino acids, and ribose for nucleotides required for repair, as well as in generation of biomass in actively dividing cells (105). Similar to the stem cells, some also consider that the growth of cancer is attributable to the presence of cancer stem cells, that upon differentiation, lose the ability to form tumors. The cancer stem cells, therefore, maintain their differentiated progeny through infinite cell divisions. The initial clue that cancer cells also rely on glycolysis was provided in the 1930 by Otto Heinrich Warburg who showed that, in a given time and under aerobic conditions, tumor tissues convert approximately tenfold more glucose to lactate than normal tissues (101). Since in presence of oxygen, the pyruvate derived from glycolysis should be used in the mitochondrial tricarboxylic acid (TCA) cycle, Warburg theorized that aerobic glycolysis results from defects in the mitochondrial oxidative phosphorylation (OXPHOS). Consistent with Warburg’s idea, cancer cells that show defects in OXPHOS due to mutations in the enzymes that drive the TCA cycle lead to the Warburg’s

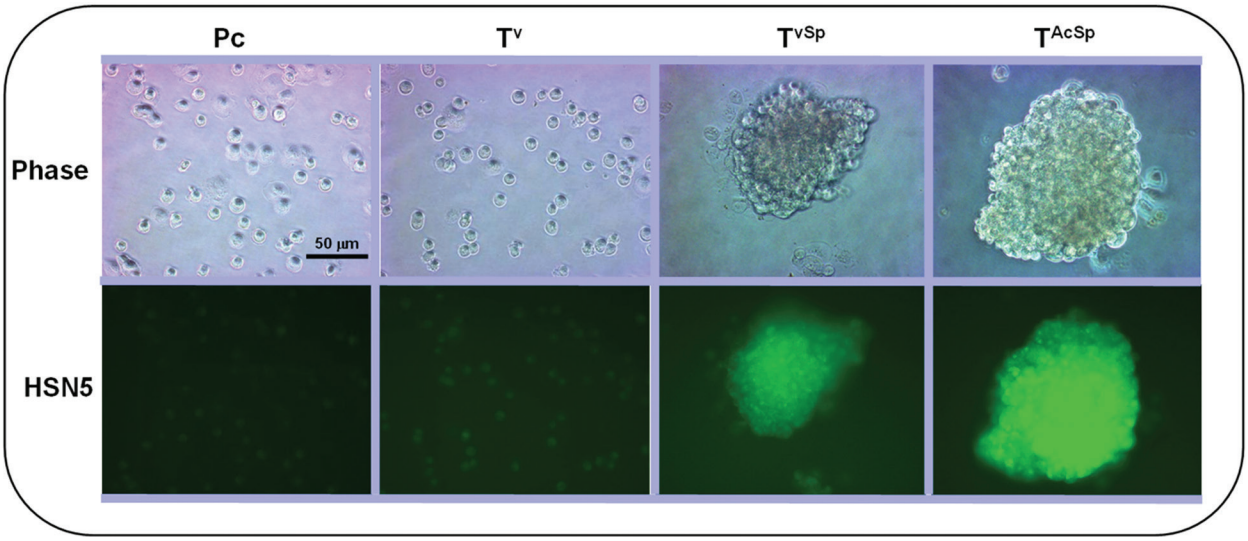

Figure 12. Hydrogen sulfide stained with HSN fluorescent probe in MDA-MB 435 damage recovered sperhoidal cells that express stemness genes (Unpublished data).

effect. However, majority of tumors fail to exhibit reduced mitochondria or mitochondrial dysfunction (226). Among other conditions that confer permanent induction of the Warburg's effect, are oncogene activation, loss of tumor suppressors, hypoxia and activation of HIF-1. For example, *SRC* oncogene activation, Oncogenic *H-Ras* and phosphatidylinositol kinase all stabilize HIF-1 leading to glycolysis under normoxia (227). Clear cell carcinomas that exhibit a loss of VHL protein or prostate cancer cells that show activation of P13K/AKT pathway through expression of *FOXO3* and stimulation of mTOR activate HIF-1 so that under normoxic conditions, these cells exhibit the Warburg effect (228).

Hydrogen sulfide maintains the stemness in the mesenchymal stem cells of the bone marrow (Liu *et al*, 2014). Bone marrow mesenchymal stem cells actively produce this gas in order to regulate their self-renewal and osteogenic differentiation. Reduced sulfhydration of cysteine residues on multiple Ca^{2+} TRP channels, causes decreased intracellular Ca^{2+} influx and results in downregulation of PKC/Erk-mediated Wnt/β-catenin signaling and defects in osteogenic differentiation of stem cells (229)(Figure 10). Consistent with the role of hydrogen sulfide in bone formation, H_2S-deficient mice display an osteoporotic phenotype that can be rescued by small molecules that release H_2S.

According to the cancer stem cell theory, any cancer cell holds the potential to become a stem cell. Such a belief is supported by a growing number of reports that show the importance of microenvironmental factors such as inflammation or damage induced signaling in inducing de-differentiation and generation of cells with a stem like phenotype (230-231). Microarray analyses show that an overwhelming number of genes that are expressed in cancers are also found during cell, RNA and DNA repair. Among the concordant genes, most notable are mismatch repair (MMR) proteins, as well as RNA and DNA repair factors (232-238). Therefore, cancer stem cells exhibit a number of phenotypic and genotypic similarities in cell behavior, signaling molecules and gene expression profile to cells undergoing repair. When the role of all genes that drive the Warburg effect is carefully examined, it becomes quite apparent that, most if not all of the implicated genes and their signaling pathways participate in repair of cell and its constituents. Genes which are integral to the glycolysis pathways including hexokinase, GAPDH and LDH-A also participate in repair and glucose uptake and glycolysis reduces hypoxia induced apoptosis (239). The evidence for repair in cancer is consistent with an on-going damage, caused by the less than optimal conditions of their microenvironment. The in-hospitable microenviroments within tumors where glucose, glutamine and oxygen have dwindling range of concentrations place a heavy burden on tumor cells that fight for survival. Regions of apoptosis within solid human cancers mark the sites where fight for survival ends with the death of cancer cells. Cells at the fringe of these death territories get damaged but nearly escape death caused either by nutritional deficiencies or hypoxia (240).

A possibility to consider is that cancer stem cells that engage glycolysis as the principal metabolic pathway are those that recover from severe damage. This damage might be induced by hypoxia, glucose deprivation, and acidosis which commonly occur in areas of tumor with poor blood flow or by hydrogen peroxide, a toxic by-product of OXPHOS (102-104)(Figure 12). To this end, *in vitro* Damage-Recovered (DR) cancer cells exhibit mitochondrial structural remodeling, and display Warburg's effect. Damage-Recovered (T^{DR}) cells also show

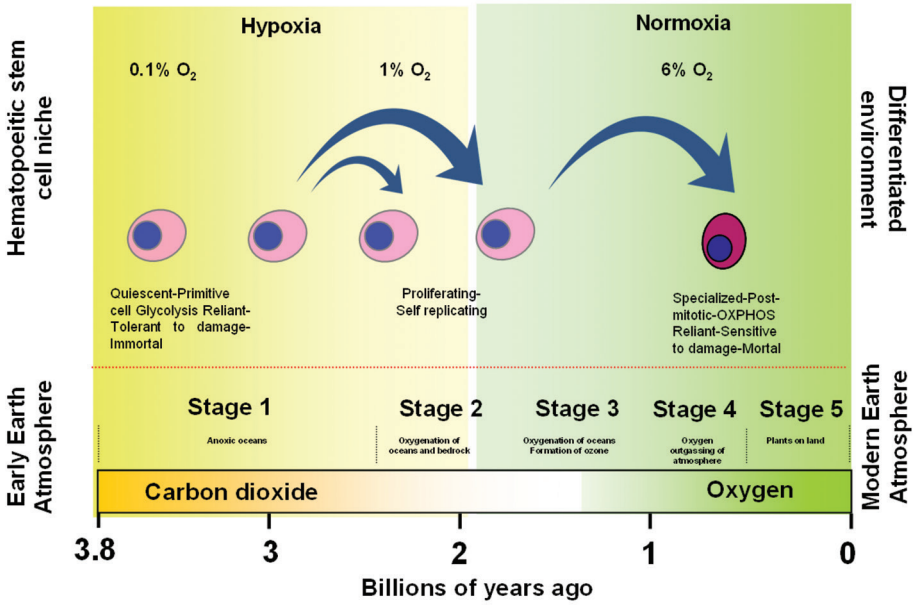

Figure 13. Comparison of niches of stem cells and differentiated cells with evolution of species.

increased aerobic glycolysis, enhanced ATP production and a high growth rate (102-104). These findings show that Warburg's effect and its consequences are induced in cancer cells that survive severe damage. Adoption of glycolysis in damage recovered cancer cells is due to up-regulation of hydrogen sulfide that drives an increase in nicotinamide phosphoribosyltransferase (Nampt), the primary enzyme that increases NAD^+ (102-104). There is also a significant correlation between the level of H_2S-Nampt and ATP production. H_2S-Nampt directs aerobic glycolysis in cancer cells that recover from damage and upon recovery it promotes their exponential growth *in vitro* and *in vivo* (102-104). Consistent with such results, the increase in hydrogen sulfide production is seen in many cancers. The endogenous production of H_2S is highly upregulated in epithelial cells of colorectal and prostate cancers, and in tumor-derived endothelial cells (241-243).

Adoption of aerobic glycolysis, which is a more primitive metabolic pathway, by cancer cells suggested to Warburg that cancer represents reversion to a primitive form of life based on a "respiratory defect". Paul Davies also recently postulated that "cancer is a type of throwback, or atavism, to an ancestral phenotype, in a way similar to how "Windows" defaults to the "safe mode" after recovering from some sort of damage (244). Since cancer is widespread among fish, reptiles, birds, and mammals, the possibility exists that cancer has deep evolutionary roots stretching back at least hundreds of millions of years and that "genome evidently comes pre-loaded with a "cancer sub-routine" that is normally idle but can be triggered into action by a wide variety of insults, such as chemicals, radiation and inflammation. Davies hypothesizes that "cancer subroutines" represent reactivation of ancient and highly conserved genes which are expressed during embryonic state as a result of insult or damage. The available evidence shows that indeed cancer stem cells arise following recovery from damage. This occurs via activating a hydrogen sulfide-Nampt switch that is critical to their rescue from damage by adoption of glycolysis, acquisition of a primitive cell phenotype, repair and long term tolerance of cells to future damage (102-104).

13. CONCLUSIONS

Life emerged on Earth, in an anaerobic environment, rich in gases emitted from volcanic eruptions that disrupted the Earth crust or changed the environment within the oceans. Early life forms not only exhibited a natural immunity towards such Earth environments but developed and even flourished in these environments. Among the gases that led to formation of life on Earth, the impact of hydrogen sulfide on sculpting life is unique since this gas likely contributed to the abiogenic synthesis of organic compounds and dramatically shaped the evolution of cells throughout their evolutionary path. It is presumed that the role of sulfide in physiological signaling is a holdover from the origin of eukaryotes and animals during anoxic and sulfidic times. Modern cells still utilize hydrogen sulfide as a signaling molecule, in pro and anti-inflammatory responses, for repair, in directing repair responses, for acquiring tolerance against damage, for modifying the mitochondrial function as well as a source of energy. Thus, hydrogen sulfide, the earliest gas that drove evolution, can simulate the properties of early life forms that provides protection against harsh microenvironmental conditions (Figure 13).

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Abbreviations: LUCA: Last universal common ancestor or cenancestor, H_2S: Hydrogen sulfide, EMP: Embden-Meyerhof-Parnas, OXPHOS: Oxidative phosphorylation, PAH: polycyclic aromatic hydrocarbon, SQR: sulfide quinone reductase, ROS: reactive oxygen species, TCA tricarboxylic acid, DR: Damage-Recovered

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