Chapter 3
Biosignatures of Cellular Components and Metabolic Activity

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Abstract The astrobiological search for biosignatures requires a working concept of the fundamental attributes of life. Life's basic capabilities of energy harvesting, metabolism, and self-replication can create objects, substances and patterns—biosignatures—that indicate their biological origins. High relative abundances of certain lipids, hydrocarbons, amino acids and polysaccharides are diagnostic products of billions of years of evolution. Lipid assemblages having narrow molecular weight ranges are key constituents of cellular membranes. The molecular structures of lipids provide details of their biosynthetic pathways. Some lipid biosignatures are diagnostic for particular groups of microorganisms. The biosynthesis of organic matter and biochemical oxidation-reduction reactions can discriminate against the heavier isotopes of carbon and sulfur and thereby create molecular isotopic patterns that indicate not only their biological origins, but also key details about biosynthetic pathways. Sulfur isotopic patterns can indicate biological redox reactions. Because microorganisms can greatly enhance, at relatively low to moderate temperatures, reaction rates between oxidized and reduced sulfur compounds, they can create a range in the stable isotopic compositions of these compounds that is substantially larger than nonbiological reactions can achieve under similar conditions. The simultaneous presence of multiple biosignature objects, substances and patterns in a demonstrably habitable earlier environment constitutes the most compelling evidence of past life.

3.1 Introduction

3.1.1 Biosignatures

The venue of exploration fundamentally determines the perspective taken for research on signatures of life, or "biosignatures". For example, because Mars and the early Earth are prominent targets of exploration for astrobiology, much research

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B. Cavalazzi, F. Westall (eds.), *Biosignatures for Astrobiology*, Advances in Astrobiology and Biogeophysics, https://doi.org/10.1007/978-3-319-96175-0_3

focuses on detecting signs of past life sequestered in the rock record of these planets. Earth's early geological record provides an opportunity to investigate the antiquity of life on Earth; it is also an instructive analogue that guides our search for biosignatures on other rocky bodies. The significance of Mars arises both from the relative similarity of Mars and Earth among the planets of the Solar System, and from the relative accessibility of the Martian geological record. It is possible to search in situ for preserved remnants of cellular components and evidence of past metabolic activity. Accordingly this chapter focuses on such features.

A biosignature is an object, substance, and/or pattern created by a biological source. The value of a biosignature is determined not only by the probability of life producing it, but also by the improbability of non-biological processes making it. The search for life beyond Earth rests upon the premise that biosignatures will be recognizable within the contexts of their planetary environments. Astrobiological investigations must therefore also consider how biosignatures might be generated, preserved, and/or detected within those contexts.

3.1.2 Concepts of Life

The search for biosignatures requires a working concept of the fundamental attributes of life. This concept helps to identify the life sustaining "services" that an environment must provide. This in turn helps to identify the most promising past or present environmental candidates for exploration. Such a working concept also helps to identify and interpret biosignatures.

Any working concept of life must admit the possibility that life elsewhere differs in fundamental ways from life on Earth. Without a second known example of life it is probably not possible to determine which characteristics are unique to terrestrial life and which are common or required for life elsewhere. Indeed some have proposed that our current perspectives might even preclude a definitive, universal concept of life (Cleland and Chyba 2007). But in order to achieve a better understanding we must start taking steps, however uncertain, along that path. Accordingly, it is productive to propose attributes of life as we know it that might be universal, as opposed to those that might represent local solutions that have been specific to survival on Earth.

National Research Council (2007) identified the following potentially universal attributes of life:

1. life must exploit thermodynamic disequilibrium in the environment in order to perpetuate its own disequilibrium state;
2. life most probably consists of interacting sets of covalently bonded molecules that include a diversity of heteroatoms (e.g., N, O, P, S, etc. as in Earth-based life) that promote chemical reactivity;
3. life requires a liquid solvent that supports these molecular interactions;
4. life employs a molecular system capable of Darwinian evolution.

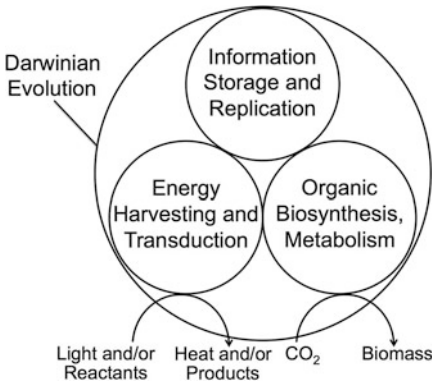

Fig. 3.1 The basic functions of life. Source: Adapted from Des Marais (2013)

These attributes implicate the following basic universal functions (Fig. 3.1):

1. life harvests energy from its environment and converts it to forms of chemical energy that directly sustain its other functions;
2. life sustains "metabolism"—a network of chemical reactions that synthesize the key chemical compounds required for maintenance, growth and self-replication;
3. life requires an "automaton," a multi-component system that is essential for self-replication and self-perpetuation (Von Neumann 1966).

Perhaps the most distinctive attribute of life is its capacity for self-replication to create populations upon which natural selection can act to maintain Darwinian evolution. Von Neumann's theory of the automaton (e.g., Von Neumann 1966) predicted the components that are essential for biological self-replication (Fig. 3.2). He had envisioned these essential components of the automaton before molecular biologists identified the actual molecular machinery of the DNA-RNA-protein system. The components required for self-replication consist of relatively complex molecules that must persist in environments so that they can function more rapidly than they are degraded. This perspective has implications regarding the unique virtues of carbon-based chemistry and it also imposes requirements on the attributes that an environment must possess in order to remain habitable.

Accordingly, life can be envisioned as a self-sustaining system that is capable of Darwinian evolution and that utilizes free energy to sustain and propagate an automaton, a metabolic network and functionally related larger structures. These functions specify a level of molecular complexity that in turn defines requirements for chemical ingredients, energy and environmental conditions that are essential to sustain life.

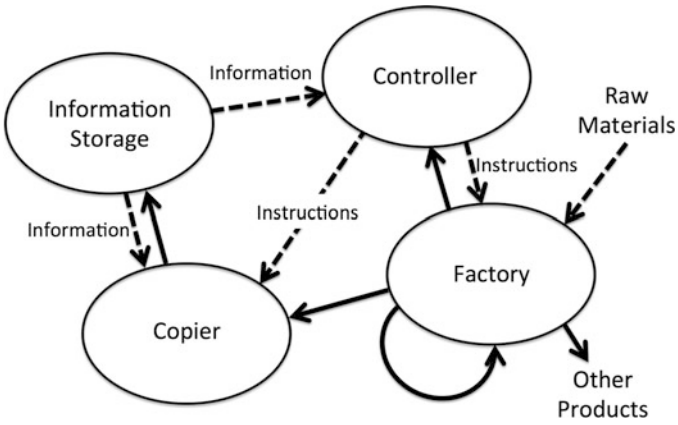

Fig. 3.2 An automaton is a system capable of self-replication and has several complex components. For example, in biological systems DNA stores and provides information (dashed arrows) and ribosome "factories" replicate other components, including proteins involved in other cellular processes. Source: Adapted from Des Marais (2013)

3.1.3 Attributes of Life

Because flight missions must make specific chemical observations to search for life, our working concept must provide more than just a short list of generic universal attributes. We can start by enumerating attributes that are universal among life as we currently understand it.

In his book "Beginnings of Cellular Life," Morowitz (1992) articulated several attributes that are shared by all life on Earth. Below are listed some key examples of these attributes and their applicability.

1. *"The chemistry of life is carried out in aqueous solutions or at water interfaces. Cells can survive the removal and restoration of cellular water, but water is essential to cellular function."* Liquid water is therefore essential to maintain the long-term survival of life.
2. *"The major atomic components in the covalently bonded portions of all functioning biological systems are C, H, N, O, P and S"*. Particular chemical compounds of these elements engage in genetic, metabolic and energy-harvesting functions and thus merit particular attention.
3. *"A cell is the most elementary unit that can sustain life."* Single-celled organisms are by far the most diverse, ancient and environmentally tolerant forms of life known. But we remain uncertain whether the earliest life was necessarily compartmentalized in cells.
4. *"There is a universal set of small organic molecules that constitutes the total mass of all cellular systems."* The set of key organic biomolecules on Earth represents an extremely small subset of all possible organic molecules. Perhaps this array is small due to the evolutionary selection for speed and efficiency of

biochemical processes. Living systems must efficiently maintain high information contents at molecular and structural levels.

5. *"Those reactions that proceed at appreciable rates in living cells are catalyzed by enzymes."* Catalysis enables cells to maintain their metabolism and to repair damage caused by environmental challenges such as radiation. Enzymes help to acquire essential energy by harvesting sunlight and by accelerating reactions between oxidized and reduced chemical species in the environment.
6. *"Sustained life is a property of an ecological system rather than a single organism or species."* For example, biofilms and microbial mats are highly successful ecological systems (e.g., Des Marais 2010). Fossilized biofilms have been identified as stromatolites in rocks more than 3.4 billion years old (Walter 1983).
7. *"All populations of replicating biological systems give rise to altered phenotypes that are the result of mutated genotypes."* All known living systems evolve by Darwinian-like natural selection. "Phenotype" is a cell's molecular and structural "machinery." Genotype is the information preserved in genomic molecules (DNA and RNA are examples in our biosphere). This interplay between phenotype and genotype is the automaton that is capable of Darwinian evolution.

This list does not necessarily preclude the possibility of radical alternatives to life as we know it. However, given the remarkable chemical and physical attributes of water and carbon compounds, together with their substantial cosmic abundances, the case can be made that at least a substantial and pervasive fraction of life elsewhere is based upon organic chemical reactions occurring in liquid water.

Basic attributes of life such as those enumerated above have allowed biochemists, paleontologists and astrobiologists to identify and interpret several categories of biosignatures in ancient geological deposits on Earth.

The following sections provide key examples of biosignatures that arise from carbon compounds and from biological redox reactions. Other categories of biosignatures include cellular microfossils (see Chap. 7), biominerals (Chap. 6) and biogenic sedimentary fabrics (Chap. 7).

3.2 Organic Molecular Biosignatures

Anomalously high relative abundances of specific organic molecules in a rock might constitute evidence of a biological origin (Sect. 3.1.3, example 4, above; Morowitz 1992). Molecules such as porphyrins, fatty acids, amino acids and polysaccharides help to sustain key biochemical functions and therefore are relatively abundant in living organisms. Certain key constituents of cellular membranes are relatively robust and can survive burial and storage in sedimentary rocks for long periods of time (more than a billion years, e.g. Brocks et al. 2005). Some of these compounds are diagnostic for particular groups of microorganisms (Sects. 3.2.1 and 3.2.2). Patterns of carbon isotopic abundances in organic compounds offer insights into metabolic processes (Sect. 3.3).

3.2.1 Membrane Polar Lipids

Membrane lipids are extremely valuable molecules for identifying living biota or its fossilized remains and have been equated to a "universal biomarker for life" (Georgiou and Deamer 2014). Indeed, membranes composed of amphiphilic lipids are the major structural components of all three domains of life—Eukarya, Archaea and Bacteria. While even relatively short chain fatty acids are good amphiphiles and readily self-assemble into micelle or monolayer structures in an aqueous environment, formation of a bilayer structure requires a hydrocarbon chain length of at least twelve carbon atoms (Deamer 1986). A biological membrane requires a more complex molecule composed of a polar head group and two long chain hydrocarbons with a C_{14} to C_{18} equivalent length capable of forming a bilayer ~40 nm thick. The hydrocarbon chains make up the membrane inner core and provide its unique hydrophobic barrier, while the polar moiety interacts with the aqueous environment and maintains cellular integrity. Such membrane amphiphiles are often referred to as intact polar lipids (IPL). They are the major component of the cytoplasmic membrane that plays a central role in energy storage and processing, both universal characteristics of life (Deamer 1997; Sojo et al. 2014).

IPL are composed of a core glycerol molecule. One hydroxyl group is attached to the polar moiety (e.g. choline, galactose) referred to as the 'head group' and the remaining two to long-chain hydrophobic moieties. A broad variety of polar head groups are universally shared throughout the three domains, however, glycerol stereochemistry, glycerol-hydrocarbon linkage and hydrocarbon chain structure vary and provide degrees of taxonomic attribution among the domains of life.

The hydrocarbon chains of Bacteria and Eukarya are acetogenic lipids composed of C2 units that derive from acetyl-CoA. These molecules are linked *via* ester-linkage to the *sn*-1 and *sn*-2 positions of the glycerol backbone. The polar head group is linked by either a phosphatide, sulfatide or glycoside linkage to the *sn*-3. Archaeal membrane lipids, aside from similar head-group architecture and fundamental amphiphilic properties, differ substantially. Archaeal IPL are composed of isoprenoid hydrocarbon chains linked by ether bonds to the *sn*-2 and *sn*-3 glycerol positions with the head group at *sn*-1. While some bacteria are known to have glycerol ether bonds, the hydrocarbon moieties are acetogenic with some limited methyl branching. Archaeal lipids are composed of C5 isoprene units that result in C_{20}, C_{25} or C_{40} chains. Biphytanyl tetraether lipids are composed of two C_{40} chains attached to a glycerol molecule at both ends that span the entire 40 nm width of the membrane. Until recently, this structural motif was thought to be unique to Archaea, but bacterial membrane-spanning lipids have now also been identified (Sinninghe Damsté et al. 2011).

Isoprenoid biosynthesis is essential for all domains of life and creates a diverse structural array of isoprene-based biomolecules—acyclic, exemplified by archaeal IPL and carotenoids, and cyclic, exemplified by the two types of triterpenoids discussed further below. There are two evolutionarily distinct pathways for the synthesis of C5 isoprene units, the mevalonate (MVA) and methylerythritol-

phosphate (MEP) pathways (see Sect. 3.2.2). Archaea use the MVA pathway to synthesize C5 isoprene units (Lange et al. 2000).

Maintaining an appropriate membrane structure is crucial to cellular integrity and function. Biological membranes are exquisitely sensitive to changes in temperature, a process termed homeoviscous adaptation (Sinensky 1974). Modification of hydrocarbon moieties is of particular importance for maintaining a gel-like state associated with cell viability. In bacteria and microeukaryotes, the hydrophobic chains are continually modified or replaced in response to environmental conditions (López-Lara and Geiger 2017). Fatty acyl chains are readily modified to prevent crystallization at low environmental temperature by shortening the acyl chains and introducing methyl branching (e.g. iso-/anteiso-) or unsaturated double bonds. At higher temperatures survival depends on the synthesis of longer acyl chains or methylation of double bonds to form cyclopropyl rings (Ernst et al. 2016). Less is known about the maintenance of archaeal membrane fluidity but it appears to occur by a somewhat different process (Oger and Cario 2013). Phase transition of highly branched isoprenoid chains occurs at lower temperatures and over a broader temperature range than in bacteria (Koga 2012). Modifications of chain structure, such as unsaturation and synthesis of C_{40} chains with cyclopentane and cyclohexane rings, also occur (Schouten et al. 2013) but they are not well understood. Environmentally triggered modifications have been described for specific archaeal genera, however, intra-genera rules do not broadly apply. Thus, while membrane-spanning C_{40} and cyclic products have been associated with survival at high temperatures, similar compounds are found in colder environments (Oger 2015).

In studies of analogue ecosystems, IPL analyses provide important information related to community composition in an environmental context and, when combined with analyses of compound-specific isotope compositions (see Sect. 3.3.2.3), they can also reveal trophic structures. Generally, The IPL structure readily degrades upon cell death (enzymatic cleavage of acyl and polar head group linkages). Thus, IPL are excellent biomarkers for extant life. However, little is known about the enzymatic degradation of the ether bond (White et al. 1996). Archaeal ether lipids are known to survive longer than bacterial ester-based lipids (Harvey et al. 1986) and have been shown to have greater potential for geological preservation (Brassell et al. 1981).

The methodology of many geochemical studies of microbial mat lipids (i.e. acid and base hydrolysis) focuses almost exclusively on free lipids (fatty acids, alcohols, hydrocarbons). Free lipids are often the products of the initial stages of diagenesis, which can partially or totally obscure the identities of the source organisms. In order to achieve a holistic perspective on biomarker diagenesis, an investigation must examine biomarkers both within living biomass and at various stages of diagenesis and preservation.

Modern microbial mats have been studied as analogs of ancient stromatolites for many years. At Exportadora de Sal, Guerrero Negro, Baja California Sur, Mexico, a wide variety of mats grow across a wide salinity gradient in an extensive network of evaporation ponds (Des Marais 1995, 2010). These ponds provide relatively stable ecosystems for the study of production, degradation and survival of biomarker lipids in microbial mats. In Pond 4 for example, over a salinity range of ~60‰ to 110‰,

Microcoleus cyanobacteria form extensive cohesive, laminated microbial mats. In order to establish the microbial community structure of this Guerrero Negro ecosystem, we initiated analysis of a full depth profile for the abundance of the acyl and alkyl IPL using Pond 4 mat cores (Orphan et al. 2008; Jahnke et al. 2008). Intervals of surface mat and underlying sediment were analysed to document changes in the bacterial and archaeal population. Archaeal lipids are not abundant in these hypersaline mats. Even in the organic-rich sediments of the lower salinity ponds, methanogenic archaea are relatively limited. IPL abundances near the mat surface indicate an almost exclusive bacterial population. Archaea are only more abundant than bacteria at the lowest terminal trophic level of the mat core system (Jahnke et al. 2008). However phylogenetic analyses revealed that archaeal diversity varied substantially at depth. Most importantly, microcosm experiments revealed that growth and concomitant changes in diversity of the archaea had occurred and could be detected by monitoring changes in archaeal biomarkers (Orphan et al. 2008).

3.2.2 Cyclic Triterpenoids

Sterols and hopanoids are two classes of cyclic triterpenoids that are both important membrane lipids, however, sterols are almost exclusively found in eukaryotes and hopanoids in bacteria. As mentioned earlier, biological membranes play a crucial role in cellular function. One of the unifying principles of thermodynamically stable membranes is the fluid mosaic model in which amphipathic globular proteins alternate among sections of phospholipid bilayer to form a viscous solution that supports permeability of the cellular boundary layer (Singer and Nicolson 1972). The physical advantage associated with the inclusion of sterols in the eukaryotic membrane is well known. Essentially any higher sterol with a planar geometry (e.g. cholesterol, ergosterol, sitosterol) will enhance lipid order and provide a more mechanically robust, flexible membrane (Lingwood and Simons 2010). Although it is still unclear whether some of the initial enzymatic steps involved for sterol biosynthesis are of bacterial origin, phylogenomics indicates that sterol synthesis was well established in the earliest of the eukaryotic lineages (Pearson et al. 2003; Desmond and Gribaldo 2009; Wei et al. 2016).

The synthesis of one molecule of cholesterol requires 11 molecules of O_2 (Summons et al. 2006). However, relatively low levels of O_2 are required (Jahnke and Klein 1983; Waldbauer et al. 2011), which is consistent with co-evolution of sterol synthesis with cyanobacteria prior to the Great Oxidation Event. Indeed, it has been suggested that the initial role of sterols in eukaryotes was protection against oxidative stress (Galea and Brown 2009). Squalene is the last common intermediate in the biosynthetic pathways for biosynthesis of sterol and hopane carbon skeletons. For sterol synthesis, the first dedicated enzymatic step is the oxygen-dependent epoxidation of squalene to 2,3-oxidosqualene (squalene monoxygenase), followed by enzymatic cyclization by oxidosqualene cyclase (OCS) to either of two C30 protosterols, lanosterol or cycloartenol. Further modifications of these molecules

result in synthesis of the structurally broad class of sterols representative of the eukaryotic kingdom (Summons et al. 2006; Nes 2011).

As noted above, hopanoids are also the cyclization products of squalene. By contrast, bacterial hopanoid synthesis is an anaerobic process. The squalene-hopene cyclase (SHC) is an ortholog of OCS with some unique properties (see Welander et al. 2012 and references therein). In contrast to the strict stereo specificity of eukaryotic OCS, bacterial SHC have very low substrate specificity (Ourisson and Rohmer 1992). It has been demonstrated in cell-free systems that SHC will accept squalene epoxide as a substrate and is not selective in stereochemistry in that both enantiomers are cyclized (Rohmer et al. 1980). SHC cyclization in bacteria results in the production of both diploptene and diplopterol and, in some bacteria, the product is tetrahymenol, a pentacyclic of the gammacerane series.

The primary hopanoid identified in natural environments is a class of complex molecules referred to collectively as bacteriohopanepolyol (BHP). BHP is synthesized by linking a polar side-chain to the hopane skeleton. A wide range of structural variations is observed in the side chain (Rohmer 1993; Talbot et al. 2007, 2008). Methylation at C-2 or C-3 of the hopane skeleton also occurs in a limited number of bacterial groups. The molecular dimensions and shape of hopanoid molecules, such as diplopterol and BHP, are similar to those of sterol molecules; this observation led Ourisson and colleagues to suggest that hopanoids in bacterial membranes were the structural equivalent of, and phylogenetic precursor to, eukaryotic sterols (Ourisson and Rohmer 1982; Ourisson et al. 1987 and references therein). The main difference in BHP or diplopterol is that the polar moiety is attached to the E ring of the hopane nucleus at C-21, whereas with sterol the hydroxyl group is located in the A ring at C-3. In vitro studies demonstrate that both diplopterol and a BHP with a tetrol side chain can order synthetic membranes in a similar manner to the order achieved by sterols (Sáenz et al. 2012; Poger and Mark 2013). In vivo physiological studies have shown that different hopanoids have distinct cellular roles, some of which clearly involve outer membrane integrity and some with yet undetermined functional roles (Welander et al. 2012). Indeed, more recent in vivo studies directly support the role that hopanoids play in outer membrane integrity by interaction with lipid A in a fashion similar to that shown for eukaryotic microdomains (Sáenz et al. 2015). Thus, similar functional roles span the bacterial-eukarya domains in meeting the demands for environmental adaptation over evolutionary time.

3.2.3 Modern Microbial Mat Ecosystems as Analogs for Life on the Early Earth

Within the context of this discussion, microbial mats are defined as organo-sedimentary structures formed by vertical, upward growth in response to burial by sediment trapping and binding, and/or encrustation by mineral precipitation and the requirement for light to fuel photosynthetic primary production. Stromatolites are

their fossil equivalents and can occur as diverse forms of layered mounds, columns, and laminated sedimentary rock fabrics throughout the Archaean and Proterozoic (Awramik 1984; Bosak et al. 2013; Pierson et al. 1992; Walter 1983). One of the earliest *bona fide* examples has been described in the 3.43 Ga Strelley Pool Chert, Pilbara Craton, Australia, within what is thought to have been a hypersaline lagoonal environment in a region of active volcanism (Walter 1994; Allwood et al. 2006). While there is no definitive evidence that these earliest Archaean mats were not formed by anoxygenic phototrophs, the massive accumulation of free oxygen likely beginning in the Neoarchaean points to an early evolution of oxygenic photosynthesis and cyanobacteria.

Early mats were widespread and abundant throughout shallow water continental shelves, lagoons and lakes, and their fossil counterparts, stromatolites, remain one of the most important indications of early life. Modern microbial mats are widely distributed globally but are more restricted environmentally. Conditions must be extreme to limit eukaryotic grazers but relatively benign for benthic microbes. Such conditions occur in numerous tidal flats, hypersaline sabkhas, polar and high latitude lakes, hydrothermal springs and various evaporative environments (e.g., Gerdes et al. 1985; Franks and Stolz 2009; Jahnert and Collins 2011; Javor 1989; Stal 2012; Vincent and Quesada 2012; Ward et al. 2012). The primary phototrophs in modern mats are cyanobacteria and the broad morphological diversity seen throughout cyanobacterial taxa are also apparent in extant mats (Allen et al. 2009; Golubic and Abed 2010; Nübel et al. 2000). In marine mats, salinity, UV tolerance and desiccation resistance are major determinants. Such environmental factors broadly define cyanobacterial diversity and, thus, mat morphology.

Highly laminated phototrophic mat communities often develop in protected marine environments with elevated salinities due, in part, to their characteristically higher evaporation rates. *Microcoleus chthonoplastes* is a cosmopolitan builder of flat, laminated marine mats (Garcia-Pichel et al. 1996) in such environments. The filaments of this cyanobacterium are composed of multiple trichomes within a common sheath. This morphological characteristic (intertwined filaments) imparts a robust, cohesive surface and the development of distinct fine laminae to the mat. Excellent examples of such cyanobacterial mats develop along a salinity gradient in evaporating ponds of the Exportadora de Sal, S.A., Guerrero Negro saltern (Des Marais et al. 1989; Des Marais 1995, 2010 and references therein). The optimal salinity range for *Microcoleus*-dominant mats lies between 75‰ and 100‰ (salinity expressed as parts per thousand). As salinity increases above 110‰, unicellular and other filamentous cyanobacteria become increasingly dominant and the mat texture becomes more gelatinous and the laminations less well-defined. At the higher salinities, endoevaporatic mats develop in gypsum crusts (Jahnke et al. 2014b). These mats are exposed to high solar irradiance throughout the day and darkness at night, which results in dramatic shifts in physicochemical gradients over mat depth and the development of distinct trophic layers, each accompanied by a complex assemblage of organic molecules.

It is generally considered that Precambrian microbial mats are the prototypical source of early petroleum reserves, whose hydrocarbon molecules form the base of

our lipid biomarker record (Peters et al. 2005). From this perspective, our knowledge of structure, function and physiological dynamics within microbial mats, particularly as they relate to development of biosignatures, such as organic molecules and stable carbon isotopic compositions, is fundamental to identifying definitively the biologic origins of stromatolites. However, organic analyses of a microbial mat entail distinct challenges. Molecules synthesized in some horizons are indeed lipid biomarkers associated specifically with various microbial groups, while others more broadly define microbes at the kingdom level. Some compounds are freely extractable (e.g. saturated hydrocarbons) and available over the full mat depth for analysis, while others that are highly functionalized (e.g. bacteriohopanepolyol) are readily entrained within the sulfide-rich matrix and form a non-extractable fraction. Many organic geochemical studies only focus on surface or near surface horizons yet, below the surface photic zone of primary production and even intermingled within, diverse groups of respiring aerobes, sulfate-reducers, fermentative bacteria and methanogenic archaea carry out continuous degradation and heterotrophic mineralization of primary organics and, in the process, contribute their own biosignatures to the mix.

Cyanobacteria in the surface layer are characterized by numerous biomarker lipids (Jahnke et al. 2004, 2008, 2014b; Palmisano et al. 1989 and references therein): IPL galactosyl-polyunsaturated fatty acid, normal chain alkanes (primarily *n*-17:1 and *n*-17:0), mid-chain branched alkanes (e.g. 7-methylhexadecane), chlorophyll *a* and numerous carotenoids (myxoxanthophyll, zeaxanthin and canthaxanthin). The presence of chlorophyll *c*, fucoxanthin, the highly branched isoprenoid C_{20} and C_{25} alkanes (HBI) and sterols indicate the presence of diatoms or other microeukaryotes. Polyunsaturated fatty acids, *n*-17:1 alkene and Chl *a*, while excellent biomarkers for vital populations, do not preserve well and decrease rapidly with depth. However, many isoprenoid lipids (HBI, sterols, carotenoids, hopanoids) survive well below the photic zone. Below the cyanobacteria a secondary layer of anoxygenic photosynthetic bacteria (*Chlorothrix, Chromatium, Rhodovulum*) harvest the residual light that penetrates into the sulfide-rich zone (Jørgensen and Des Marais 1986). Biomarker lipids for these phototrophs include phospholipid-derived fatty acids $\Delta 11\text{-}C_{18:1}$ and cyclopropyl-C_{19}. Distinct lipophilic pigments (γ-carotene, spirilloxanthin, bacteriochlorophyll *a*, and Bchl *c* represent the purple and *Chloroflexus*-like bacteria in this horizon (Klappenbach and Pierson 2004; Oren et al. 1995; Palmisano et al. 1989; Pierson et al. 1994).

Many lipid biomarker molecules have been documented in various organic geochemical mat studies (Abed et al. 2008; Allen et al. 2010; Boon et al. 1983; Boudou et al. 1986; Bühring et al. 2009; Grimalt et al. 1992; Jahnke et al. 2008, 2014a; Pagés et al. 2014a, b; Rontani and Volkman 2005), but generally their fates have not. For instance, in the Guerrero Negro *Microcoleus* mats, the carotenoid/chlorophyll ratio increased with depth (Palmisano et al. 1989), indicating that Chl *a* had decomposed relative to carotenoid substantially over a timescale of months. For Camargue mats, the cyanobacterial biomarkers $n\text{-}C_{17:1}$, $n\text{-}C_{17}$ and monomethylalkane (MMA) all decreased rapidly below the surface layer with only slightly greater survival potential documented for the saturated C_{17} and MMA within the deeper anoxic horizon

(Wieland et al. 2008). A similar pattern has been observed for the Guerrero Negro *Microcoleus* mat (Jahnke et al. 2014a). Hopanoids, particularly BHP, appear to be ubiquitous components of microbial mats. As noted above, numerous bacteria synthesize hopanoids. BHP is often most abundant in the surface layer and generally decreases with depth (Jahnke et al. 2014b; Pagés et al. 2014a). However, in other mats BHP appears to be synthesized primarily at depth by the anaerobic community (Blumenberg et al. 2013). Archaeal isoprenoid IPL were present in much lower abundances compared with bacterial fatty acids in the GN *Microcoleus* mat (Jahnke et al. 2008). Archaeol was the primary IPL over the entire core depth of 130 mm although a GDGT-C_{40} isoprenoid represented a relatively greater proportion in the anoxic layers.

Studies of lipid biosignatures in modern microbial mats help to establish ecological relationships, identify the diversity of biosignatures that might be preserved, and also understand the processes of taphonomy that have shaped the organic molecular fossil record.

3.2.4 Linking Geological and Biological Records

Cyclic triterpenoid molecules are valuable for understanding the evolution of functional adaptation in microorganisms. Accordingly, they help to elucidate the organic geochemical record in rocks. The cyclic skeletons of these molecules are resistant to diagenesis and catagenesis. Hopanes and steranes, defunctionalized products of catagenesis, are perhaps the two most important categories of organic biomarkers, and both have substantial records dating back to the 1.64 Ga Barney Creek Formation in northern Australia (Brocks et al. 2005; Brocks and Schaeffer 2008). Beyond this age, attempts to identify *bona fide* syngenetic structures have been plagued by contamination with younger organics and the highly mature nature of available samples (French et al. 2015). Major advances in technologies for analysis and the protocols for recovery of kerogen-bound syngenetic biomarkers have set the stage for a more comprehensive exploration of ancient rocks for evidence of early life. In this respect, open-system catalytic hydropyrolysis (HyPy) has been essential (Love et al. 1995). On the biological front, great progress has also been made in answering questions related to source organisms, environmental distribution and physiological functions. This is attributable largely to new molecular tools that allow for identification of the genes responsible for the biosynthesis of cyclic triterpenoids (Welander et al. 2010, 2012).

A case in point is the evolved history of hopanoid biomarkers. The first discoveries of hopanoid source organisms exclusively involved bacterial genera that were facilitated by some form of aerobiosis (obligate, microaerobic, faculative). All of the strict anaerobes and archaea initially analzyed had no hopanoids (Rohmer et al. 1984). As a consequence, hopanoids became popular indicators for environmentally available O_2. However, hopanoid biosynthesis does not require O_2 and we are now aware that some strict anaerobes indeed synthesize hopanoids in anoxic environments

(Blumenberg et al. 2006; Fischer et al. 2005; Härtner et al. 2005; Sinninghe Damsté et al. 2004). At the time of this writing, hopanoids have still not been found in archaea. The identification of the 2-methyl homologue of BHP in numerous cyanobacteria further spurred the proposal that the 2-methylhopane prevalent in the geological organic record was a biomarker for cyanobacteria (Summons et al. 1999). However, the subsequent identification of 2-methyl-BHP in an anoxygenic phototroph, *Rhodopseudomonas palustris*, somewhat diminished the usefulness of 2-methyl-BHP as a cyanobacterial biomarker (Rashby et al. 2007). With the identification of the 2-methylase gene (HpnP) in *R. palustris* by Welander et al. (2010), the phylogenomic interrogation for HpnP identified both cyanobacteria and two groups within the Alphaproteobacteria (the *Rhizobiales* including *R. palustris* and *Methylobacterium*) as the likely evolutionary source. However, based on the limited number of genomes then available, the precise branching order could not be definitively established (Welander et al. 2010). More recently, increased availability of HpnP-containing genomes has allowed Ricci et al. (2015) to establish that C-2 methylation arose within a group of *obligately aerobic* alpha-proteobacteria. Thus, 2-methyl-hopanes identified in contemporary environments or sedimentary rocks are still biomarkers for the presence of O_2, but cannot be considered specific to cyanobacteria.

The complexities of interpretation of the organic sedimentary rock record are numerous and, in many cases, require multiple lines of evidence. The recovery of abundant quantities of hopanes from the geological record emphasizes the importance of BHP as a bacterial biomarker and has stimulated investigation of the molecular variability of the side chain moiety. In particular, the analysis of BHP structures from numerous bacteria and recent sediments by the groups of Rohmer (2008 and references therein) and Talbot et al. (2007, 2008) have established the detailed structures and molecular distributions of many BHP molecules, and in some cases, provided insights into the sources and environmental contexts of geohopanes (Farrimond et al. 2000, 2003). As noted above, some hopanoids are methylated at C-2 and others at C-3 of ring A, but these are limited in distribution. Molecular structures in combination with the carbon isotopic compositions of individual biomarkers can further refine attribution. Diplopterol and/or diploptene are common in all hopanoid-producing bacteria and are the easiest to analyze, however, quantitatively they are not generally significant (Rohmer et al. 1984; Eickhoff et al. 2014). This is not the case for the 2-methyldiplopterol-producing alphaproteobacteria. Diplopterol and its 2-methyl homologue can be quantitatively significant in this group, while the synthesis of the 2-methyl homologue of BHP generally is not (Knani et al. 1994; Vilcheze et al. 1994; Renoux and Rohmer 1985; Bravo et al. 2001; Welander et al. 2010). Additionally, in *R. palustris* and *Bradyrhizobium* spp., tetrahymenol, its 2-methyl homologue and their hydrocarbon derivatives, gammaceranes, are also abundant (Bravo et al. 2001). In the geological record, gammacerane is considered the diagenetic product of tetrahymenol (Ten Haven et al. 1989) and thus the identification of co-occurring methylgammacerane and methylhopane would provide taxonomic attribution to this group.

As noted above, sterol biosynthesis can occur at very low O_2 levels (Jahnke and Klein 1979, 1983), the O_2 K_m for the squalene monooxygenase is 70 nM (Jahnke

and Klein 1983), and synthesis has been demonstrated at levels as low as 7 nM (Waldbauer et al. 2011). Such O_2 requirements are consistent with levels near the base of the oxic-anoxic chemocline of a cyanobacterial mat, or roughly 10^{-2} to 10^{-3} present atmospheric levels (PAL). Thus cyanobacterial mats and the ecosystem that they support provide a viable scenario for the evolution of this biosynthetic pathway. As witnessed by the stromatolite record, these microbial mats were plausibly the first environments with available biogenic O_2.

Organic biomarker molecules are primarily derived from cellular lipids. Such lipid biomarkers have been valuable for characterizing source rocks in petroleum science (Peters et al. 2005), which has until recently formed the basis of our knowledge for understanding the organic geological record. Indeed the assertion that biohopanoids are 'more abundant globally than any other group of natural products' with an estimated abundance of 10^{12} tons (Ourisson and Albrecht 1992) has led to a fruitful exploration for these molecules in the biological record. Investigating the generation and preservation of geohopanoids is now central to understanding Earth's organic record and its potential for detection of past or present life on Mars (Eigenbrode 2008). Biohopanoids are subject to a wide range of early diagenetic reactions, including loss or alteration of functional groups, structural modification and rearrangements. C_{31} to C_{35} geohopanes are the most abundant in sedimentary organics and record BHP molecules (Peters et al. 2005). C_{30} geohopanes that represent simple hopanoids (e.g. diploptene, diplopterol) are much less abundant. Major degradation of organics is carried out by aerobic microbes, however, under such conditions, modification of the pentacyclic skeleton of BHP and C_{30} molecules is limited and no alteration to the BHP linear side-chain occurs (Tritz et al. 1999). In recent anoxic environments, sulfurization is thought to play a major role in the preservation of organic biomarkers. Inorganic sulfur species (e.g. H_2S) react with functionalized biolipids such as the polyol side chain of BHP during the early stages of diagenesis and form organic sulfur compounds, namely thiophenes (Sinninghe Damsté and de Leeuw 1990) that remove these molecules from further bacterial degradation. Relatively strong chemical degradation is required to release bound organosulfur compounds (OSC) from the macromolecular structure and not always with satisfactory results. However the development of open-system catalytic hydropyrolysis (HyPy) resolves many of these issues and allows release of covalently bound biomarker molecules as their defunctionalized homologues with minimal structural rearrangement (Love et al. 1995). Indeed, HyPy is also well-suited to the analysis of older, catagenically matured rocks, and has become an essential tool for characterizing syngenetic organic material bound in the overmature kerogens in Archaean rocks (Bishop et al. 1998; Brocks et al. 2003; Marshall et al. 2007).

3.3 Stable Isotope Abundance Patterns as Biosignatures

3.3.1 Stable Isotope Basics

Several elements that are abundant in biological materials have two or more stable isotopes. The stable isotopes of an element differ in the number of neutrons in their nuclei. This difference in nuclear mass has minimal effects on the atoms' electron orbitals but it creates differences in the vibrational energy states of their polyatomic molecules. Molecules having heavier isotopes have lower vibrational energy states than those having lighter isotopes, creating differences in the molecules' physical properties and in the isotopic compositions of suites of molecules at mutual chemical equilibrium. Molecules having heavier isotopes have slightly more stable bonds and therefore tend to react more slowly. Accordingly, biochemical processes can affect the stable isotopic compositions of reactants and products in ways that differ from those caused by non-biological processes. Such differences form a basis for distinguishing between biosignatures and the products of other processes.

In the natural sciences, the stable isotopic composition of a sample is typically expressed as the ratio of the abundance of the lighter isotope over that of the heavier isotope, relative to a standard. For example, carbon isotopic abundances are represented as follows:

$$\delta^{13}C_{PDB} = \left(\left({}^{13}C/{}^{12}C \right)_{sample} / \left({}^{13}C/{}^{12}C \right)_{PDB} \text{-}1 \right) 1000 \tag{3.1}$$

$\delta^{13}C_{PDB}$ is the difference in permil (parts per thousand) between a sample and carbon isotopic standard (the Peedee Belemnite carbonate—PDB). Herein we will use the terms $\delta^{13}C_{org}$ and $\delta^{13}C_{carb}$ to indicate $\delta^{13}C$ values of organic matter and carbonates, respectively. Sulfur isotopic abundances are represented as follows:

$$\delta^{34}S_{CD} = \left(\left({}^{34}S/{}^{32}S \right)_{sample} / \left({}^{34}S/{}^{32}S \right)_{CDT} \text{-}1 \right) 1000 \tag{3.2}$$

$\delta^{34}S_{CD}$ is the difference in permil between a sample and the Canyon Diablo Troilite meteorite sulfur standard. Analogous equations express isotopic compositions for nitrogen ($^{15}N/^{14}N$, air nitrogen standard), hydrogen (D/H of 'standard mean ocean water'—SMOW), and oxygen (e.g., $^{18}O/^{16}O$, SMOW).

3.3.2 Carbon Compounds and Microbial Metabolism

The objective is to determine whether and how patterns of isotopic compositions among suites of carbon compounds might indicate biological activity and, perhaps, even identify particular microbial populations. The approach is first to understand

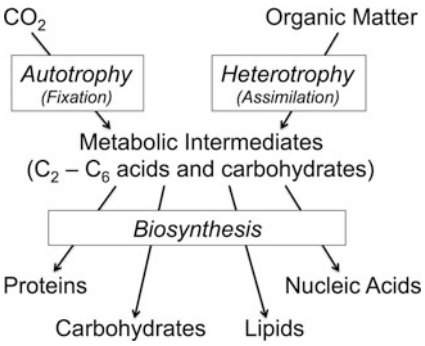

Fig. 3.3 Biosynthesis in autotrophic and heterotrophic organisms. Source: Adapted from Hayes (2001)

how enzymatic reactions that are centrally important for life can play major roles in determining isotopic compositions of the major classes of biochemicals, e.g., carbohydrates, proteins, nucleic acids and lipids. Figure 3.3 provides an overview of the relevant processes.

Enzymes create reaction products whose isotopic compositions can serve as isotopic signatures of those enzymes. In the absence of life, rates of organic reactions are typically very sluggish under ambient conditions. Enzymes generally accelerate organic reaction rates substantially, as noted above (*"Those reactions that proceed at appreciable rates in living cells are catalyzed by enzymes"* Sect. 3.1.3 example 5). In kinetically controlled chemical reactions such as these, the isotopic compositions of reaction products reflect the compositions and molecular configurations of particular intermediate chemical species in the reaction pathways. Because at least some of these intermediate species have elevated vibrational energy states, they are thereby enriched in the lighter isotope(s) relative to the reactants. The magnitude of the differences in isotopic compositions between reactants and products is related to the isotopic discrimination that occurs during the reaction. The magnitude of this discrimination can, in turn, help to identify particular reaction intermediates and thereby serve as biosignatures of particular enzymatic reaction mechanisms. Ultimately, the reaction networks that synthesize and recycle these compounds can create patterns of molecular isotopic abundances that reflect these metabolic pathways. Such isotopic patterns can thereby become biosignatures.

Biologically-mediated molecular isotopic patterns among biomolecules can reflect certain basic attributes of life (Morowitz 1992): *"There is a universal set of small organic molecules that constitutes the total mass of all cellular systems,"* and *"Those reactions that proceed at appreciable rates in living cells are catalyzed by enzymes."* Such patterns are useful biosignatures to the extent that they can be distinguished from patterns created by any non-biological processes.

The following discussion explores the following examples that illustrate how carbon isotopic patterns can be interpreted as biosignatures: (1) isotopic discrimination during the assimilation of carbon (autotrophy), (2) isotopes in intermediary metabolism, (3) lipid isotopic compositions, (4) isotopic patterns within molecules, and (5) isotopic contrasts between reduced and carbonate carbon in ancient sedimentary rocks.

Table 3.1 Isotopic discrimination by major carbon fixation pathways (Hayes 2001; House et al. 2003)

Enzyme/pathway	E, ‰
RuBisCO, C3 pathway (algae)	30
RuBisCO, C3 pathway (bacteria, cyanobacteria)	22
Acetyl CoA pathway	15–36
Reductive TCA cycle	4–13
3-Hydroxypropionate cycle	0–4

E (epsilon) is the difference, in permil, between the instantaneous isotopic compositions of products and reactants in the carbon fixation reactions

3.3.2.1 Isotopic Discrimination by Autotrophic Carbon Fixation

The biological fixation of small molecules like CO_2 is typically accompanied by significant isotopic discrimination of the fixed carbon. The magnitude of this fractionation establishes the isotopic relationships between autotrophs and dissolved inorganic carbon (DIC) and atmospheric CO_2.

The following carbon fixation pathways are particularly prominent among microorganisms: (1) reductive pentose phosphate cycle (Calvin-Benson-Bassham cycle), (2) reductive tricarboxylic acid (rTCA) cycle, (3) 3-hydroxypropionate cycle, and (4) reductive acetyl-CoA pathway (Preuss et al. 1989; House et al. 2003). These pathways exhibit systematic differences in the magnitude of the isotopic discrimination that accompanies carbon fixation (e.g., Table 3.1).

Ribulose Bisphosphate Carboxylase-Oxygenase (RuBisCO) is the principal enzyme utilized for carbon fixation by cyanobacteria, algae and plants. Today, RuBisCO is by far the quantitatively most important enzyme for fixing CO_2 (e.g., Dhingra et al. 2004; Raven 2013). In geologically recent sedimentary rocks, isotopic discrimination by primary producers is principally responsible for creating a 20 to 30 permil difference (e.g. Hayes et al. 1999) between the $\delta^{13}C_{PDB}$ values of organic and carbonate carbon. The $\delta^{13}C_{PDB}$ values of recent marine carbonates lie typically in the range between -2 and $+3$ permil. In the modern biosphere this isotopic dichotomy between reduced carbon and carbonates is a biosignature for biological carbon fixation.

In Earth's early biosphere isotopic discrimination during carbon fixation has probably contributed substantially to maintaining the dichotomy in isotopic compositions between sedimentary organic matter and coeval carbonates (e.g., Hayes et al. 1983, Hayes 1993; Des Marais 2001). But it is interesting to inquire whether carbon fixation pathways other than the Calvin cycle were relatively more prominent earlier in Earth history. Later in this chapter, this topic will be addressed by examining the record of carbon isotopic compositions of reduced carbon and carbonates in Earth's ancient sedimentary rocks.

3.3.2.2 Isotopic Fractionation Within Intermediary Metabolism

The arrows depicted in Fig. 3.3 indicate the net carbon flows in a cell that is growing and building biomass. The isotopic compositions of the major components of cells (carbohydrates, proteins, nucleic acids, lipids) reflect the outcome of isotopic competition between the enzymatic reactions that lead to these components (Hayes 2001). Changes in the physiological state of a cell can, in some cases, shift the relative abundances of these cellular components. Hayes (2001, 2004) provides comprehensive reviews of carbon isotopic discrimination associated with biosynthetic processes.

3.3.2.3 Isotopic Compositions of Individual Lipids

The production of biomass is associated with numerous variables that depend upon the specific organism, its growth substrate and its environment. In this respect the use of stable isotopes provides a valuable tool for further characterization of organic material. In this section we will address the role of compound specific isotopic analyses (CSIA) of carbon in individual lipid molecules, in particular, in distinguishing source and determining metabolic status. Section 3.3.3 discusses further the broader topic of bulk stable isotopes for biogeochemical analysis.

Organisms preferentially use the lighter isotope of carbon (^{12}C), which results in fractionation between the substrate (heavier or ^{13}C enriched) and product (lighter or ^{13}C depleted biomass). This is generally the rule but as discussed below, bulk isotope effects for a few organisms can result in ^{13}C enriched biomass. Factors that determine the ^{13}C content of individual organic molecules follow similar kinetic processes within the biosynthetic networks that distribute ^{12}C and ^{13}C throughout cellular systems (see Hayes 1993, 2001 for a detailed discussion). The complementary use of structurally diagnostic molecules, together with their particular C-isotopic compositions, can refine biomarker attribution to specific physiologies and metabolic groups of organisms.

The lipid biomarkers discussed above are the products of two biosynthetic pathways that result in carbon skeletons that are either linear (acetogenic) or multibranched (isoprenoid). The biosynthetic pathway for acetogenic lipids, such as fatty acids, derives from acetyl-CoA and is fundamentally similar in all organisms (Reilly 2016). Some exceptions in bacteria are the synthesis of branched fatty acids (primarily iso- and anteiso-) and anaerobic synthesis of unsaturated fatty acids (Cronan and Thomas 2009), whereas in eukaryotes, oxidative desaturation results in the synthesis of both monounsaturated and polyunsaturated fatty acids with a few bacterial exceptions, including cyanobacteria (Murata et al. 1992; Cook and McMaster 2002).

Isoprenoid biosynthesis is far more complex. The synthesis of the universal C5-isopentenyl diphosphate (IPP) subunit occurs by two different mechanisms (Fig. 3.4). In the well-known mevalonate (MVA) pathway, IPP carbon is derived

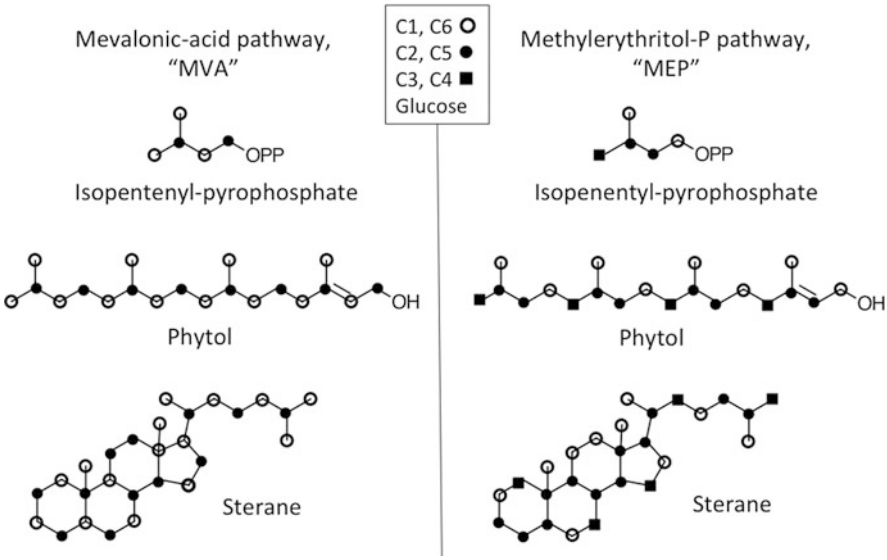

Fig. 3.4 Sources of carbon atoms in isopentyl-pyrophosphate (IPP), phytol and sterane for the mevalonic acid (MVA) and methylerythritol-P (MEP) pathways. In the MVA pathway all five IPP carbon atoms derive from C1 and C2 atoms of glucose. For MEP, one carbon atom in IPP derives from C3/C4 of glucose. Source: Adapted from Hayes (2001)

from acetyl-CoA, while in the more recently described methylerythritol phosphate (MEP) pathway, the substrates are pyruvate and glyceraldehyde-3-phosphate. From the initial discovery of the MEP pathway by Rohmer (2008) and his colleagues, extensive work has demonstrated that this new mechanism for IPP synthesis functions in bacteria and plant plastids. Most organisms only use one of these pathways for isoprene synthesis, however plant cells also use the MVA pathway for IPP biosynthesis in their cytoplasm. The MVA pathway appears to be the ancestral pathway for biosynthesis of IPP in archaea and the cytoplasm of non-photosyntheyic eukaryotes (Lange et al. 2000). The second stage of isoprenoid synthesis commences with condensation of the C5-isoprene subunits to generate linear polymers of defined chain lengths (C_{20}, C_{25}, C_{30}, C_{40}, i.e. C_{5n}). Cyclization results in a vast number of biomolecules of geochemical importance (Peters et al. 2005). From the perspective of CSIA networks, it is important to note that, in plant cells and particularly in algae, exchange of cytosolic- and plastid-isoprenoid precursors can and does occur (Vranová et al. 2012) providing a potentially additional level of complexity to the interpretation of algal biomarker lipids. The isotopic consequences of biosynthesis, carbon source and discrimination, and the added dimension of intracytoplasmic compartmentalization in algae result in significant differences among isoprenoids in phototrophic eukaryotes (see Hayes 2001; Pearson 2014 for synopsis and discussion).

Distinct differences in the carbon isotopic compositions of acetogenic and isoprenoid lipids, relative to bulk organic carbon, were first noted in the geological

record by Logan and colleagues (Logan et al. 1995, 1997). They suggested that transition in biota between the Proterozoic and Phanerozoic was marked by a major change in the carbon isotopic relationships among bitumen, associated lipids and syngenetic kerogen. These observations have stimulated discussions related to the likely causes (Close et al. 2011; Pawlowska et al. 2013) that rely on available CSIA for lipid biomarkers, preservation of lipids and likely depositional environments, and point to the importance of continued efforts to understand ^{13}C discrimination within cellular products in environments of potential relevance to interpretation of the ancient organic record.

3.3.2.4 Carbon Isotopic Abundance Patterns Within Molecules

A distinctive characteristic of the biosynthesis of organic molecules is the intramolecular carbon isotopic patterns developed by the flow of precursor molecules through reaction networks. Individual carbon atoms of a carbohydrate molecule can be either enriched or depleted relative to the average molecular composition (see Hayes 2001 for full discussion related to all compound classes). Similar consequences are expected regarding the intramolecular isotopic compositions of lipid molecules and regarding their effects upon the $\delta^{13}C$ values of biomarkers.

For any class of compound, carbon isotope abundances will ultimately reflect initial fractionations associated with carbon assimilation (see 3.3.2.1), metabolic carbon flow (Figs. 3.3 and 3.4) and distinct fractionation events associated with enzymatic reaction mechanisms. As noted above, *n*-alkyl carbon skeletons are essentially acetate polymers derived from acetyl-coenzyme A. The general ^{13}C-depletion noted for fatty acids relative to bulk carbon in most organisms arises primarily due to a fractionation associated with the enzymatic decarboxylation of pyruvate to form acetyl-CoA (DeNiro and Epstein 1977; Monson and Hayes 1980). Fractionation is localized in the carboxyl position of acetyl-CoA and is carried through the isotopic order in biosynthesis of the fatty acid molecule. Additional discriminations may further be associated with the individual sources and sinks for acetyl-CoA in individual organisms.

The isoprene carbon skeleton is constructed from isopentenyl-pyrophosphate subunits. However, as noted above, IPP can be biosynthesized by two different mechanisms (Rohmer 2008). Characterization of intramolecular ordering depends heavily upon ^{13}C labeling experiments and NMR analysis (Eisenreich et al. 2004). As with *n*-alkyl synthesis, acetyl-CoA is the fundamental subunit for biosynthesis of IPP *via* the MVA pathway. Three molecules of acetyl-CoA are used to synthesize mevalonate; decarboxylation results in IPP. The consequence is incorporation of three carbons derived from the methyl position and two carbons from the carboxyl position (Fig. 3.4). Thus MVA isoprenoids are expected to be somewhat enriched relative to the accompanying acetogenic lipids.

For the MEP pathway, the IPP subunit is biosynthesized by condensation of one molecule of pyruvate and one of glyceraldehyde 3-phosphate to form 1-deoxyxylulose-5-phosphate. The mechanism involves transfer of an acyl anion synthon by thiamine

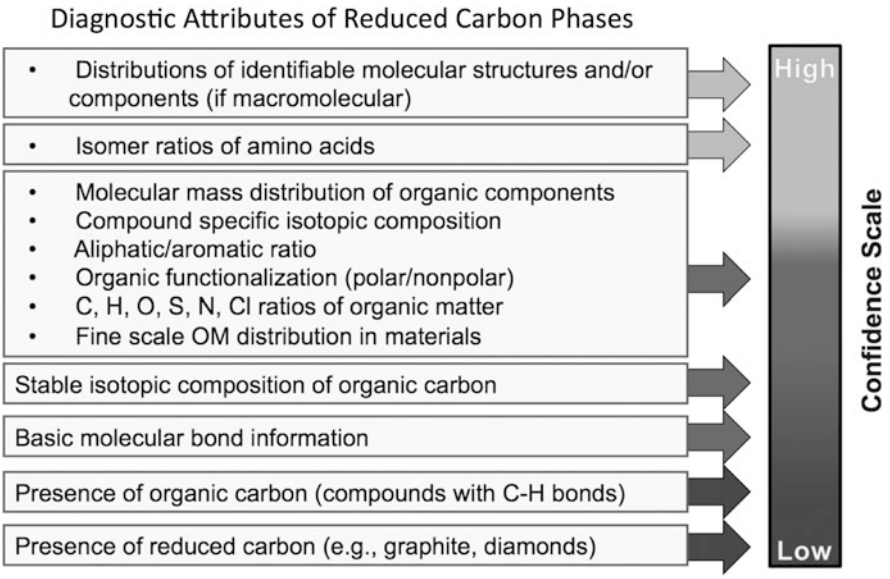

Fig. 3.5 The confidence in establishing the origins of reduced carbon and its compounds increases with the extent to which compound classes, specific molecular structures and stable isotopic compositions are characterized. Source: Adapted from Mustard et al. (2013)

pyrophosphate (activated acetaldehyde) to glyceraldehyde-phosphate. The reaction mechanism of the transketolase is very similar to other enzymes such as pyruvate dehydrogenase and pyruvate carboxylase to which specific fractionation is attributed (Eisenreich et al. 2004; Hayes 2001, 2004). The resulting IPP molecule reflects the contribution of glucose carbon atoms C1–C2 and C1–C2–C3, respectively. Intramolecular isotopic analyses indicate that IPP-C3 is depleted in ^{13}C compared to the other atoms (Schouten et al. 2008). The isotopic difference for the IPP-C3 *via* the MVA pathway was found to be significantly smaller. Accordingly different pathways for IPP biosynthesis produce IPP having different intramolecular isotopic distributions (Fig. 3.4), but ultimately these differences must be assessed within the context of metabolic sources for intermediary carbon and the reaction networks (Tang et al. 2017).

3.3.3 Reduced Carbon and Carbonates in Sedimentary Rocks

The discussions above indicate how our confidence in establishing the origins of reduced carbon and its compounds increases with the extent to which compound classes, specific molecular structures and stable isotopic compositions can be characterized (Fig. 3.5).

It should be noted, however, that processes such as microbial diagenesis, oxidation, radiation and elevated temperatures and pressures can degrade organic matter during its burial and storage in sedimentary rocks. The typically low abundances of diagnostic compounds in ancient rocks poses analytical challenges and is aggravated by the effects of contamination. These difficulties become more severe in older Precambrian rocks, particularly those from the Archaean Eon, because they have typically experienced greater degrees of alteration than younger rocks. Accordingly, whereas reduced carbon occurs in sedimentary rocks of all ages, organic molecules and their isotopic patterns tend to be restricted to younger, less altered sedimentary rocks.

3.3.3.1 Carbon Biogeochemical Cycles and the Ancient Rock Record

The fidelity with which isotopic composition of sedimentary organic carbon can be established as a biosignature requires that we understand the environmental context of the carbon inventories and processes that have shaped Earth's oceans, crust and interior. These components interact *via* the biogeochemical carbon cycle, an array of carbon reservoirs linked by a network of physical, chemical and biological processes. The overall carbon cycle actually consists of multiple nested cyclic pathways that differ with respect to some of their reservoirs and processes (Fig. 3.6; Des Marais 2001). However, all pathways ultimately pass through the hydrosphere and atmosphere, and it is this common course that unites the entire carbon cycle and allows even its most remote constituents to influence our environment and biosphere.

3.3.3.2 Early Isotopic Record in Reduced Carbon and Carbonates

Figure 3.6 illustrates schematically the biologically induced dichotomy in isotopic compositions between reduced carbon reservoirs *versus* carbonates. The near-identical ranges of $\delta^{13}C$ values of biomass and recent sedimentary organic matter indicate the biological origins of reduced carbon in sedimentary rocks. Thermal alteration of sedimentary organic matter tends to increase the $\delta^{13}C$ values of the residual kerogens (e.g., Hayes et al. 1983), therefore metamorphism likely accounts for the shift in the range of $\delta^{13}C_{org}$ values observed between the reservoirs of sedimentary organic matter *versus* the reduced carbon in metamorphic rocks (e.g., Des Marais 1997). The isotopic dichotomy persists in sedimentary rocks despite these effects, in part because the $\delta^{13}C_{org}$ values of kerogen, a highly refractory form of reduced carbon in ancient sedimentary rocks, are remarkably resistant to alteration (Des Marais 1997). This dichotomy spans more than 3.5 billion years (e.g., Schidlowski 1988; Fig. 3.7) and is perhaps the oldest, most widespread evidence of early life preserved in the geologic record.

As a cautionary note, carbonaceous chondrite meteorites also exhibit carbonates that have $\delta^{13}C$ values that are greater than those of co-occurring reduced carbon phases (Pearson et al. 2006). The similarity of these patterns to those in Earth's

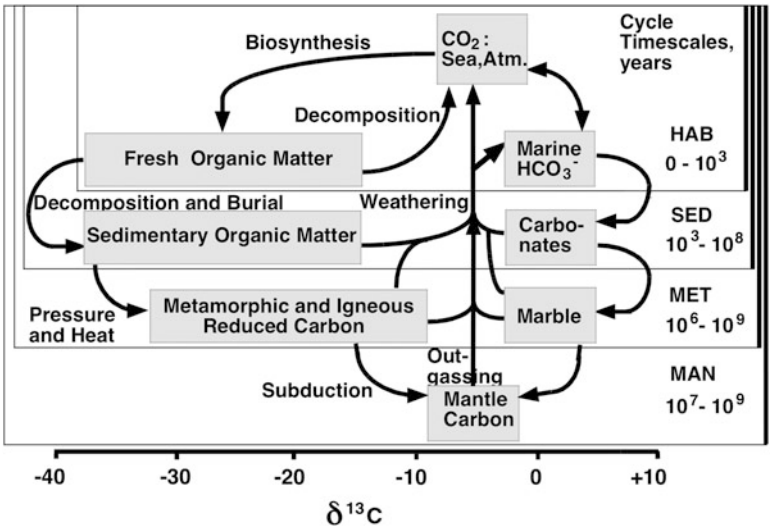

Fig. 3.6 Biogeochemical C cycle, showing principal C reservoirs (boxes) in the mantle, crust, oceans and atmosphere, and showing the processes (arrows) that unite these reservoirs. The range of each of these reservoir boxes along the horizontal axis gives a visual estimate of the $\delta^{13}C$ values most typical of each reservoir. The vertical bars at the right indicate the timeframes within which carbon typically completely traverses each of the four carbon sub-cycles (the "HABitable, SEDimentary, METamorphic and MANtle" sub-cycles). For example, C can traverse the hydrosphere-atmosphere-biosphere (HAB) sub-cycle typically within a time scale between 0 and 1000 years. Source: Adapted from Des Marais (1997)

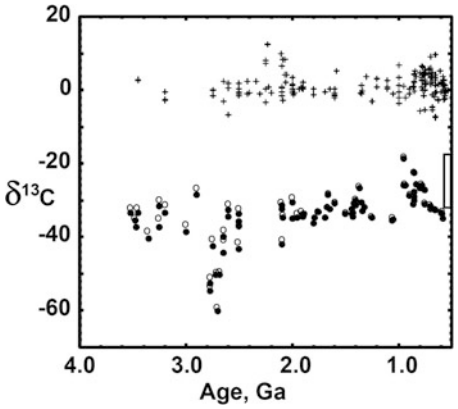

Fig. 3.7 Plot of age *versus* δ_{carb} (crosses) and δ_{org} (circles) for Archaean and Proterozoic kerogens. Kerogen data (filled circles) are corrected for the effects of thermal alteration (Des Marais 1997). Uncorrected data are shown as open circles. The $\delta^{13}C_{org}$ range for Phanerozoic kerogens is indicated by the narrow box along the right margin of the figure. Source: Adapted from Des Marais (1997)

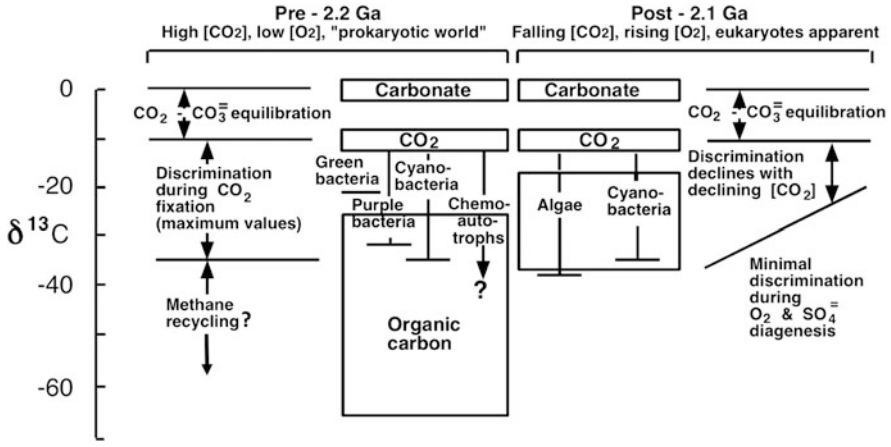

Fig. 3.8 Ranges of $\delta^{13}C$ values of carbonates, seawater CO_2 and sedimentary organic carbon, together with the processes proposed to explain their distribution prior to 2.2 Ga and subsequent to 2.1 Ga. The arrows associated with the various groups of autotrophic bacteria and algae illustrate the maximum isotopic discrimination expected for each group. The sloped line on the right depicts declining discrimination over time, perhaps in response to declining CO_2 levels in the environment. Source: Adapted from Des Marais (1997)

sedimentary record illustrates that interpretations of carbon isotopic patterns must be supported by other geochemical data and observations about paleoenvironments and associated processes. Such supporting evidence has been obtained for Earth's early geologic record (e.g., Hayes et al. 1983; Schopf and Klein 1992; Schidlowski 1993), however analogous data are still needed in order to interpret the carbon isotopic record on Mars.

Figure 3.8 represents schematically the long-term trends in the range of $\delta^{13}C_{org}$ and $\delta^{13}C_{carb}$ values observed through hundreds of analyses. In kerogens older than 2.2 Ga, $\delta^{13}C_{org}$ values are widely scattered, ranging from values around −25 to as low as −65, with many values more negative than −35. In contrast, $\delta^{13}C_{org}$ for kerogens younger than 2.1 Ga lie in the narrower range −25 to −35; virtually no values are more negative than −36. The range of $\delta^{13}C_{org}$ values typical of Phanerozoic kerogens is somewhat more positive (Fig. 3.7). The range of $\delta^{13}C_{carb}$ values typically lies within a few permil of 0, except ca. 2.2 billion years ago, and also during the Neoproterozoic.

For the ca. 3.2–3.5 Ga volcano-sedimentary sequences in the Kaapvaal (-South Africa) and Pilbara (Australia) cratons, $\delta^{13}C_{carb}$ averages 0(+/−2) ‰ (Veizer et al. 1999), and $\delta^{13}C_{org}$ ranges from −25 to −41‰ (Fig. 3.6; Strauss and Moore 1992; Des Marais 1997). Such $\delta^{13}C_{org}$ values are consistent with the notion that early Archaean ecosystems were driven by autotrophy. Such values are conventionally interpreted to indicate discrimination by the pentose phosphate (Calvin) cycle operating under conditions of high CO_2 that favor maximum isotopic discrimination (Fig. 3.8; e.g., Schidlowski 1993). However, the broad $\delta^{13}C_{org}$ range observed for these early Archaean sequences is reminiscent of the wide range of discrimination

exhibited during autotrophic C assimilation by diverse microorganisms, anaerobes in particular (Table 3.1). Therefore, the carbon isotopic record of early Archaean carbonates and kerogens also might indicate that anoxygenic photoautotrophic bacteria, chemoautotrophic microorganisms and methanogens contributed substantially to global primary productivity.

Sedimentary carbon isotope abundances might also indicate the environmental consequences of biological activity. As noted above, in sedimentary rocks younger than 2.1 Ga, $\delta^{13}C_{org}$ values lie in the range −20 to −35 and virtually no values are more negative than −36 (Figs 3.7 and 3.8), consistent with contributions from various photoautotrophs. However, in rocks older than 2.2 Ga, $\delta^{13}C_{org}$ values range from −25 to as low as −65. $\delta^{13}C_{org}$ values <−37‰ require contributions from strongly ^{13}C-depleted biomass, which has been attributed to the consumption of methane (see Eigenbrode and Freeman 2006; Eigenbrode et al. 2008 and references and discussions therein). Extremely low $\delta^{13}C_{org}$ values ca. 2.7 Ga might indicate the prevalence of biogeochemical methane cycling in oxidant-deficient closed basin depositional environments (Flannery et al. 2016). Consumption of ^{13}C-depleted methane by either anaerobic archaeal consortia or aerobic methane oxidizers, or by both of these, provides mechanisms for the production and transport of highly ^{13}C-depleted organic matter to sedimentary environments (Hayes 1994; Hinrichs et al. 2000). The absence of very negative $\delta^{13}C_{org}$ values after 2.1 Ga might indicate that such environments had become more oxidized by either oxygen or sulfate. The proposal that sulfate levels in the deep ocean became substantial during the Mesoproterozoic (Canfield 1998) is consistent with this carbon isotopic evidence.

The $\delta^{13}C_{org}$ and $\delta^{13}C_{carb}$ record is also consistent with the oxidation of the Proterozoic environment between 2.3 and 2.0 Ga (Fig. 3.8). A large positive $\delta^{13}C_{carb}$ excursion between 2.2 and 2.06 Ga (Fig. 3.7; Baker and Fallick 1989; Karhu and Holland 1996) indicates that the relative rate of organic burial increased. Increased net organic burial would have led to increased $[O_2]$, $[SO_4^{=}]$ and sedimentary $[Fe^{3+}]$. Oxic respiration and bacterial sulfate reduction became, as they are today, globally dominant pathways for organic utilization and decomposition. Notably these pathways express minimal carbon isotopic discrimination (e.g., Blair et al. 1985; Kaplan and Rittenberg 1964), thus, they apparently created few opportunities to create sedimentary organic C having $\delta^{13}C_{org}$ values lower than −35, consistent with the post-2.1 Ga $\delta^{13}C_{org}$ record (Fig. 3.7). In the post-1.9 Ga world, isotopic discrimination associated with the CO_2-assimilation by aerobic photoautotrophs became the dominant mechanism controlling the dichotomy between $\delta^{13}C_{org}$ and $\delta^{13}C_{carb}$.

The search for carbon isotopic evidence of life in rocks older than 3.5 Ga is challenged both by their rarity and by metamorphism. Claims of potential biosignatures have been consistently challenged by counterclaims questioning their validity. Examples include the debate surrounding the origin and significance of isotopically light reduced carbon phases in Archaean quartzose rocks at Akilia, Greenland (Mojzsis et al. 2002; Friend et al. 2002; Fedo and Whitehouse 2002). Some postulate that mantle processes contribute isotopically light carbon phases to

crustal rocks (e.g., Horita 2005; Horita and Polyakov 2015). These debates underscore the need to establish the actual age when any potential biosignatures were emplaced in a deposit and whether the environment was indeed habitable at that time (e.g., Rosing and Frei 2004). The report of potential biosignatures in early Archaean hydrothermal vent precipitates (Dodd et al. 2017) is a recent example of efforts to adopt this approach.

3.3.4 Isotopic Patterns Arising from Biological Redox Reactions

The relationships between patterns of stable isotopic composition and biological activity can also be illustrated by the energy requirements of life (Fig. 3.1): "Life must exploit thermodynamic disequilibrium in the environment in order to perpetuate its own disequilibrium state" (e.g., National Research Council 2007; Sect. 3.1.2). Enzymes harvest energy by accelerating reactions between oxidized and reduced chemical species ("redox reactions") in the environment (e.g., oxidation of reduced C, S, and N compounds). "Those reactions that proceed at appreciable rates in living cells are catalyzed by enzymes" (Morowitz 1992; Sect. 3.1.3). Life can capture energy by catalyzing redox reactions to exceed rates that would occur in the absence of life.

3.3.4.1 Sulfur

Sulfate reducing bacteria (SRB) obtain energy by coupling the reduction of sulfate to sulfide with the oxidation of organic matter or H_2 (Postgate 1984). Laboratory cultures of SRB incubated at ambient temperatures discriminate against ^{34}S, generating sulfides having $\delta^{34}S$ values that range typically between 10 to 40 permil lower than the $\delta^{34}S$ of sulfate when its abundance is in excess of the amount consumed (e.g., Canfield 2001). Theoretical calculations indicate that, under near-ambient conditions, the $\delta^{34}S$ values of sulfates and sulfides in isotopic equilibrium can differ by several 10s of permil. In the absence of biological activity, the sulfate oxyanion is strongly kinetically inhibited against achieving isotopic equilibrium within the temperature range required to achieve such large $\delta^{34}S$ values. Therefore, if the depositional environment can be constrained, the differences in $\delta^{34}S$ between sulfates and sulfides can become potential biosignatures and also record changes in SRB activity and environmental conditions over time.

Figure 3.9 illustrates $\delta^{34}S$ values for the 3.5 billion-year record of sedimentary sulfides. $\delta^{34}S$ values for sulfides older than 2.5 Ga typically lie within a few permil of 0, a range that is also typical of $\delta^{34}S$ values of mantle-derived peridotites and pyroxenites (Chaussidon and Lorand 1990). However, ~3.49 Ga sedimentary sulfides from the Dresser Formation in Western Australia exhibit $\delta^{34}S$ values that are

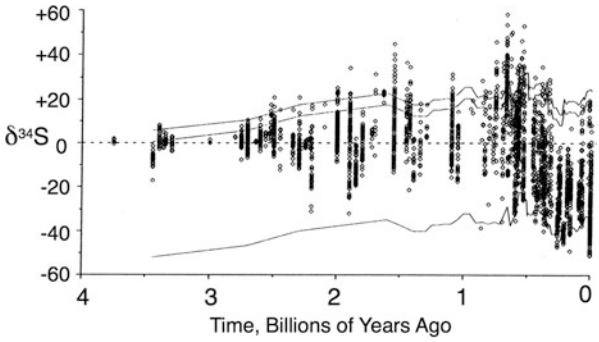

Fig. 3.9 Sulfur isotopic compositions of sulfides in ancient sedimentary rocks. Source: Adapted from Canfield (2001)

some 20 permil lower than values of coexisting sulfates (Shen et al. 2001). The authors interpreted these $\delta^{34}S$ values to indicate biologically driven sulfur transformations. The significant increase in the range of $\delta^{34}S$ values of sedimentary sulfides at ~2.3 Ga documents a substantial increase in the magnitude of isotopic fractionation by microorganisms (Canfield 2001). This change probably reflects an increase in seawater sulfate concentrations, consistent with an associated increase in the oxidation state of the global surface environment (Canfield 2001).

3.3.4.2 Metals

Stable isotope abundances of biologically important metals have also been explored as biosignatures. For example, iron isotopes have received considerable attention during the past fifteen years (e.g., Johnson et al. 2008a). The most substantial differences between various iron species are due to isotopic discrimination that occurs during changes in the redox state of iron or between iron-bearing species having different bonding states (e.g., between different iron complexes, etc.). In near-neutral pH waters where Fe^{2+} is the most mobile dissolved iron species, the $^{56}Fe/^{54}Fe$ value of Fe^{2+}_{aq} is low relative to the corresponding value of Fe^{3+} in iron-bearing minerals. Microbially mediated Fe^{3+} reduction produces the greatest abundances of isotopically distinct iron, compared to quantities produced by any non-biological processes. The quantities of iron cycled across redox boundaries were much larger in the Archaean than at present and were due to the much larger pools of Fe^{2+} and Fe^{3+} that interacted in these ancient environments. Isotopic evidence for the expansion of microbial Fe^{3+} reduction during the late Archaean is consistent with the increased production of large inventories of Fe^{3+} and organic matter (Johnson et al. 2008b). Very likely this increased production was driven by the expansion of photosynthetic communities. The magnitude of the isotopic signature of Fe^{3+} reduction in sediments decreased between ca. 2.4 to 2.2 Ga, perhaps due to the ascent in global prominence of microbial sulfate reduction which, in turn, decreased the availability of reactive iron for Fe^{3+} reduction. Research to interpret stable isotopes of iron and other metals offers a promising future both for environmental geochemistry (e.g., Wiederhold 2015) and for astrobiology.

3.4 Final Comments

The extraordinary complexity of organic chemistry can record an enormous volume of information that can be interpreted to reveal the origins and processes of organic deposits. Analyses of preserved organic molecular biosignatures can reveal not only the former presence of life but also key details about diverse physiologies and ecosystems. Stable isotopic biosignatures provide complementary information about processes and environmental conditions.

The relevance of organic biosignatures has now been firmly extended to Mars exploration, given the discoveries of organic matter in Martian meteorites (e.g., McKay et al. 1996) and in Gale Crater, Mars by the Curiosity rover (e.g., Freissinet et al. 2015). The ongoing challenge is to locate organic matter that has been sufficiently well preserved (see Summons et al. 2011) to retain key attributes (Fig. 3.5) that could indicate its origins. Recent plans for a proposed Europa lander mission include a search for potential organic biosignatures (Hand et al. 2017).

Other categories of biosignatures (metal isotopes, morphologies, minerals and biofabrics) are still vitally important. The simultaneous presence of multiple biosignature objects, substances and patterns in a formerly habitable environment would constitute the most compelling evidence of life.

Acknowledgements NASA Ames Research Center and the NASA Astrobiology program supported the preparation of this chapter. This work is dedicated to the memory of John M. Hayes—scholar, mentor and lifelong friend.

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