

2. THE ORIGIN OF CELLS

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Ideally, a treatise that claims to be focused on cellular evolution would give substantial coverage to the earliest stages of life. Unfortunately, the cumulative effects of nearly four billion years of chemistry, physics, and geology have erased all traces of precellular life. As a consequence, we will probably never be able to trace with certainty the earliest steps in the emergence of life from an inorganic world. This has not prevented the growth of an active field of “origin-of-life” research, one of the goals of which is to combine our understanding of the physical sciences and biochemistry to identify the most plausible scenarios for launching the Earth into the age of biology.

Now thousands of cells thick in some places and diversified into millions of species, the Earth’s biological skin has been molded from the beginning by historical contingencies, most notably the unique mix of elemental resources that make up the planet. The laws of chemistry and physics further dictated how these elements could be organized into biology’s structures and functions. Evolution is opportunistic, with all changes reflecting processes involving “descent with modification,” and only a tiny fraction of imaginable evolutionary changes have occurred. The nature of the genetic machinery that happened to evolve at an early stage, combined with the basic rules of population genetics, dictates what pathways remain open to evolutionary exploitation today.

Life on Earth is a peculiar mix of ingredients – nucleotides, amino acids, carbohydrates, and lipids, relying on just a small subset of the possible types of these building blocks. A rare element, phosphorus, is essential in energy and information transmission, and transition metals like iron, zinc, manganese, nickel, copper, and molybdenum are widely used in the catalytic cores of proteins. One of life’s oddest features is the reliance on proton pumping to generate return gradients for ATP formation. Are these universal features of cellular biochemistry inevitable necessities of life, or might they simply reflect the specific conditions under which life first arose, i.e., the legacy of the singular successful lineage that gave rise to all other species on the planet?

Living systems differ from inanimate objects in several key ways: 1) an ability to acquire and convert energy and material resources into new organic compounds – metabolism and growth; 2) a reliable mechanism for storing information and converting it into a phenotype – genetics and individuality; and 3) a means for transmitting information and biotic materials from one generation to the next – reproduction and inheritance. The temporal order by which these three features emerged remains unclear. However, once they were simultaneously present in enough

individuals to avoid extermination by vagaries in the environment, a permanent platform was in place for the most powerful force in the natural world – evolution by natural selection, biology’s intrinsic mechanism for designing and refining the very features of life. Given a population of individuals, some level of variation among individuals, and a mechanism of heritable transmission of phenotypic differences across generations, natural selection is an inevitable property of life. Thus, if we desire a singular time point for the origin of life, a logical defining event is the origin of evolution by natural selection.

But herein lies the problem. How did life get to the point where an ability to evolve by natural selection (indeed, an inability to avoid such a process) was locked in forever? Did metabolism arise before genetics, providing the fuel for the emergence of the genetic machinery (necessary for heritable variation), or vice versa? Once a genetic system was established, how was it faithfully maintained across generations? And at what point did membranes (necessary for individuality) come in?

THE EARLIEST STAGES

An understanding of the early features of the geosphere, combined with an appreciation of the peculiar shared molecular attributes of today’s organisms, provides a logical basis for narrowing down the broad range of possible first steps toward the origin of life. Although most of those who think about such matters implicitly assume that life initiated on Earth, there is no formal basis for rejecting the hypothesis that the seeds of the biosphere were derived from another planet. Whether or not this is the case, we are still confronted with the same questions about life’s origin.

We know that the Earth originated ~4.6 billion years ago (BYA), and was then sporadically bombarded with substantial interstellar debris for one to two billion years (Sleep et al. 1989; Nisbet and Sleep 2001; Bottke et al. 2012; Johnson and Melosh 2012). Some of the more massive impacts generated enough heat to sterilize the entire planet. Thus, it is generally thought that the roots of life must be younger than 3.8 BYA. However, biology might have experienced a number of false starts prior to this point, as small graphite inclusions (with carbon isotope ratios compatible with a biological origin) have been found in rocks dating to 4.1 BYA (Bell et al. 2015).

Further refinement of this key point will not be easy. Rock formations dating earlier than 3.5 BYA are extremely rare, and the first universal common ancestor of life most likely was so simple that no fossils were produced. However, organic signatures pointing to biological activity have been found in rocks from 3.8 to 3.4 BYA (Rosing 1999; Furnes et al. 2004; Tice and Lowe 2004), and the oldest known fossils, some from filamentous organisms and others potentially eukaryotic, date to 3.5 to 3.2 BYA (Schopf 1993; Rasmussen 2000; Schopf et al. 2002; Knoll 2004; Wacey et al. 2011; Brasier et al. 2015).

Singular events like the origin of life do not lend themselves to testing any aspect of evolutionary theory. Nonetheless, our understanding of biology’s molecular building blocks (e.g., amino acids, nucleotides, and lipids) would be advanced if their production could be linked to contexts in which life might have first emerged.

Identifying such scenarios requires an understanding of bioenergetic, geochemical, and physical opportunities and constraints. Given, for example, that carbon in the early Earth's atmosphere was dominated by oxidized forms (CO_2 and CO resulting from volcanic outgassing), some source of sustained external energy would have been required for the construction of reduced-carbon compounds upon which all life is built. In addition, given the absence of atmospheric oxygen, there would have been no ozone shield, and hence the damaging effects of UV light at the Earth's surface would have been tens to thousands of times greater than today (Cnossen et al. 2007). This suggests that life likely arose in an energy-rich, photo-protective setting. Some sort of structured environment would also have been essential as a means for colocalizing the interacting molecules necessary for some semblance of individuality.

The most celebrated experiments showing that simple forms of organic matter can be generated in abiotic environments are those of Miller (1953; Miller and Urey 1959). Based on the assumption that the earliest atmosphere harbored methane, ammonia, hydrogen, and water, the four compounds were sealed into a sterilized glass apparatus and subjected to cycles of electrical discharges followed by cooling periods to condense the resultant products. A few days of such treatment revealed the synthesis of abundant quantities of urea, sugars, formaldehyde, hydrogen cyanide, among other things. Reanalysis of the generated residues decades later revealed all of the amino acids used in today's organisms (Johnson et al. 2008).

Simple biochemical reactions underlying the results of these experiments are known. For example, starting with the one-carbon compound formaldehyde (CH_2O), through a series of steps involving water, hydrogen cyanide, and ammonia, the Strecker reaction (Figure 2.1) yields the spontaneous production of the simplest amino acid glycine. Similar reactions starting with more complex aldehydes (having side residues to the $-\text{CHO}$ group other than the H in formaldehyde; Figure 2.1) lead to other amino acids (Benner et al. 2010). Carbohydrates are much less stable than amino acids, but are stabilized and differentially channeled towards pentoses such as ribose (essential to RNA) when in complex with borate (Ricardo et al. 2004; Kim et al. 2011). Thus, ribose might have accumulated in environments enriched with borate salts.

Although the Miller-Urey experiments reinforced the popular idea that life emerged spontaneously out of a "primordial soup" (Haldane 1929; Oparin 1938), a number of doubts have been raised about the prebiotic-soup hypothesis (Maden 1995). The most prominent problem is that the early atmosphere was likely much more oxidative than the one imposed by Miller and Urey, with CO_2 (rather than methane) being the primary carbon source and N_2 (rather than ammonia) the primary nitrogen source (Zahnle et al. 2010). A second issue is that open water would be counterproductive to the maintenance of the organic aggregates essential to the nucleation of life. And finally, there is the matter of how long life could have relied on abiotically generated carbon sources before experiencing a resource-limitation crisis. Carbonaceous meteorites are known to harbor numerous organic compounds, including most of the metabolites within the citric-acid cycle deployed by most of today's organisms (Cooper et al. 2011), but it is unclear that such sporadic delivery could provide the sustenance for emerging life forms.

These and other doubts about a "heterotrophy-first" origin of life have inspired

several alternative hypotheses focused on settings conducive to the sustained geological production of organic molecules. Under these “autotrophy-first” hypotheses, the synthesis of simple organic compounds is fueled by continuous sources of energy rather than by sporadic bursts of atmospheric electricity, and water is viewed as an aid, not a hindrance. Although there are still unresolved issues, the important unifying feature of all of these models is the view that life initially became established in the absence of information-bearing polymers. Under this view, there was a prebiotic phase of autotrophic metabolism, with a genetic system somehow arising secondarily and gaining regulatory control of energy harvesting.

One of the earliest autotrophy-first scenarios was proposed by Wächtershäuser (1988, 1997, 2007), who envisioned a chemoautotrophic origin of life in a high-pressure, high-temperature environment associated with underwater volcanic activity, an idea first broached by Baross and Hoffman (1985). In the presumed presence of one-carbon compounds such as CO, CO₂, COS, HCN and CH₃SH, and with iron (and nickel) sulfides acting as catalysts, a sort of primitive form of carbon fixation was postulated to be driven spontaneously. A specific reaction that Wächtershäuser had in mind was the oxidation of FeS with H₂S to produce FeS₂ (pyrite), and releasing hydrogen ions (protons) and electrons. The products of this reaction were proposed to drive the reduction of CO₂ to formate (HCO₂), and then to more complex chemical species such as acetate (CH₃CO₂) and pyruvate (CH₃COCO₂).

Two attractive features of this model are that pyruvate is a primary participant in the major metabolic cycles of today’s organisms (e.g., Figure 2.2), and that metal-sulfur clusters serve as the catalytic centers of numerous metabolic enzymes. Indeed, Wächtershäuser went so far as to suggest the possibility of the fixation of carbon by the entry of CO₂ into a reverse form of the citric-acid cycle (whose forward reaction is used in the breakdown of sugar in today’s organisms; Figure 2.2). Under his model, the hypothesized intermediate metabolites are thiol analogs (containing -SH groups) of the usual players, with H₂S (rather than the usual H₂O) entering at various steps, as this is thought to provide a more kinetically favorable setting for running the citric-acid cycle in reverse. Under this primordial form of metabolism, energy transduction would have been accomplished by thioesters rather than by the phosphate-bearing molecules (e.g., ATP) essential to today’s organisms. Thus, it may be no coincidence that a core of today’s metabolism operates in a phosphate-independent manner using enzymes enriched in iron-sulfur complexes (Goldford et al. 2017).

The existence of modern hydrothermal-vent communities of microbes dependent solely on a continuous flow of chemistry and energy demonstrates the ability of such environments to support life. However, this need not imply that the current microbial inhabitants of such environments have been derived in a linear line of descent from the time of life’s origin. Nevertheless, some aspects of Wächtershäuser’s model have been validated (Cody 2004). Laboratory experiments imposing hydrothermal vent-like conditions have yielded pyruvate in the presence of transition-metal sulfides (Cody et al. 2000), and reactions involving pyruvate can lead to more complex organic molecules, some of which have properties related to the lipids essential to building membranes (Hazen and Deamer 2007). Novikov and Copley (2013) found that at temperatures much more compatible with life (25 to 110°C), pyruvate reacts with H₂S, H₂, and NH₄ to yield products such as aldols, lactate, alanine, propionic

acid, and sulfur-containing organics, the specific blends being strongly dependent upon temperature and the mineral substrates. Starting with the metabolites of glycolysis and the pentose-phosphate pathway (Chapter 18), Keller et al. (2014) observed almost the full set of reactions of these pathways in a completely abiotic setting, and Muchowska et al. (2019) found that a mixture of pyruvate and ferrous iron yields 9 of the 11 intermediate metabolites of the citric acid cycle (all but citrate and oxalosuccinate in Figure 2.2).

The key point here is that metabolites generated by abiotic processes are themselves subject to downstream chemical conversion to a large number of alternative compounds, with specific catalysts channeling reactions down specific pathways. Under the metabolism-first view, this raises the intriguing possibility that the basic structure of many of today's metabolic pathways are reflections of primordial sets of abiotic chemical reactions that fueled life's origin, with enzymes emerging secondarily as catalytic enhancers for pre-existing reactions.

Regardless of what one thinks of the details, Wächtershäuser's attempt to draw a connection between inorganic chemistry, geology, and the roots of biology inspired a new generation of hypotheses for the catalytic origin of life by geothermal forces, as reviewed in Cody (2004) and Stüeken et al. (2013). Although a ringing endorsement cannot be given to any one of these ideas, the focus is now on narrowing down the rich pool of candidate settings to the one(s) most conducive to the origin of life. Notably, almost all current hypotheses for the origin of life view the colonization of open marine waters occurring only after the establishment of ion-tight membranes, quite a different scenario than inferred by the primordial-soup hypothesis.

Two particularly plausible scenarios are outlined below (Figure 2.3), each based on a geological setting in which a freely available, energy-capturing reaction shares key features with metabolic mechanisms in today's organisms. The most notable aspect of such models is the implication that the origin of life is not just a chance event with an infinitesimally small probability, but an essentially unavoidable consequence of the early Earth's geochemical and atmospheric properties. If this view is correct, then life has very likely emerged on other Earth-like planets, perhaps even incorporating similar metabolic processes.

The alkaline hydrothermal-vent hypothesis. The famous “black smokers” emerging from oceanic hydrothermal vents provide an obvious source of geothermal energy and chemistry for scenario envisioned by Wächtershäuser (Corliss et al. 1981). However, their short longevity, extremely low pH, and lack of compartmentalization prompted questions over their suitability as cradle-of-life candidates (Martin and Russell 2003; Lane et al. 2010; Russell et al. 2014). Less extreme variants include lower-temperature (40-90°C), alkaline hydrothermal-vent systems (Russell and Hall 1997; Martin et al. 2008; Russell et al. 2010). In such settings, ocean fluids percolate deep into the Earth's crust, where they interact with iron compounds to release hydrogen, a strong electron donor and hence a source of energy in the presence of suitable electron acceptors. Playing the latter role, dissolved CO₂ entering from above is reduced to simple hydrocarbons such as methane, formate, and acetate. N₂ is also reduced to NH₃, and sulfates to H₂S, in such environments, providing potential paths for the downstream integration of nitrogen and sulfur into organic compounds. Moreover, such vents support the growth of porous towers of calcium

carbonate up to tens of meters in height, providing potential sites of compartmentalization necessary for the origin of individuality.

Like Wächtershäuser, the proponents of the alkaline hydrothermal-vent hypothesis postulate an initially purely geological mechanism of carbon fixation that was somehow eventually supplanted by evolved biotic mechanisms of energy generation (Lane and Martin 2012), and there are other attractive aspects of this sort of setting. First, alkaline hydrothermal vents present a 10,000-fold proton gradient, as the pH for the ocean-water influent is $\simeq 6.0$ while that for the effluent is $\simeq 10.0$. Such a setting is analogous (and some would argue a direct antecedent) to the peculiar mechanism by which almost all of today's cells produce energy – a proton gradient across biological membranes used to drive ATP production by chemiosmosis (Foundations 2.1). Second, the same metal-sulfide groups (involving Fe and Ni) envisioned as operating in early abiotic metabolism comprise the catalytic sites for energy transfer in the electron-transport chain deployed in today's organisms (Hall et al. 1974; Cammack et al. 1981).

Notably, one of the five known mechanisms of carbon fixation in today's organisms, the acetyl CoA (or Wood-Ljungdahl) pathway, has similarities to the pathway invoked by Martin and Russell (2003), in particular a reaction between CO_2 and hydrogen. The acetyl CoA pathway is the only known carbon-fixation mechanism that yields energy (driving ATP synthesis) in the process of reducing carbon. It is deployed by two distantly related groups of anaerobic prokaryotes – the archaeal methanogens and the bacterial acetogens, both of which obtain all of their C, N, and S resources from simple gases – CO_2 , CO, H_2 , N_2 , and H_2S . These two lineages deploy apparently unrelated enzymes in the acetyl CoA pathway and produce different final products (methane vs. acetate), raising the intriguing question as to whether they reflect an ancient episode of parallel evolution (Lane and Martin 2012). Although two independent origins of a similar mechanism for extracting energy and organic material from inorganic gases may appear highly implausible, as will be noted below, there are a number of other ways in which bacteria and archaea carry out similar functions with apparently unrelated molecules.

The terrestrial geothermal-field hypothesis. As enticing as the previous hypothesis may seem, a marine setting need not have been essential for the origin of life. Some have argued that terrestrial hydrothermal fields (hot springs and geysers) associated with volcanic activity harbor most of the advantages envisioned by the hydrothermal-vent hypothesis as well as others (Mulkidjanian 2009; Mulkidjanian and Galperin 2009; Mulkidjanian et al. 2012a; Damer and Deamer 2015). Under this alternative view, life would have originated in shallow ponds, with solar irradiation being the primary energy source. In contrast to the sodium-rich conditions associated with marine environments, the condensates found in such ponds likely would have been enriched with potassium, metal sulfides, zinc, and boron, with the concentration of phosphorus possibly being $> 100\times$ that at hydrothermal vents. Assuming regularly occurring wet/dry cycles, concentrations of most other reagents would likely have been elevated as well, further enhancing the likelihood of self-assembly.

One of the many mysteries in cell biology is the widespread use of potassium relative to sodium. Intracellular concentrations of potassium, zinc, manganese, and

phosphate (and most other elements; Chapter 18) are generally higher than those found in modern sea water, whereas the opposite is true for sodium (Mulkidjanian et al. 2012b). To achieve these disparities in ion concentrations, modern cells invest considerable energy in the operation of ion pumps (Chapter 18). Thus, the high potassium/sodium ratio expected under the geothermal-field hypothesis provides a potential explanation for the origin of this cellular feature – the earliest membranes almost certainly would have been permeable, and ions with the greatest environmental availability would arguably have been more subject to exploitation in the earliest stages of cell-physiological evolution.

There are other attractive features of the geothermal-field hypothesis. First, although today's terrestrial geothermal fields have extraordinarily low pH levels, this would likely not have been the case on an early Earth devoid of oxygen, as the H_2S gas associated with geothermal activity would not have been oxidized to sulfuric acid. Second, metal sulfides likely would have precipitated in shallow waters. ZnS crystals are powerful photocatalysts, raising the possibility that diverse hydrocarbons might have been produced by a sort of abiotic photosynthesis in such shallow, light-rich environments. Third, as they are highly efficient at scavenging UV light, ZnS and MnS crystals also have photo-protective capacity. Thus, Mulkidjanian and colleagues envisioned a layered system, with production of organics occurring at the surface, and the lower layer harboring protocells for harvesting such molecules. In accordance with the geothermal-field hypothesis, phylogenetic analyses of the ages of various enzymes (based on first appearance in the Tree of Life) suggest a very early appearance of those utilizing Zn, Mn, H_2S , and K (but not Na) (Dupont et al. 2010; David and Alm 2011).

Deamer and Georgiou (2015) provide a synopsis of the empirical evidence supporting the hydrothermal-vent vs. the geothermal-field hypotheses, and propose key tests for further discrimination between the two. However, the scenarios painted by these two hypotheses are by no means the only possible routes to biotic evolution. Notably, although both hypotheses are focused on environments with fairly high temperatures, a number of experiments with RNA have shown that the assembly and maintenance of polymers is actually facilitated at low temperatures (reviewed in Higgs and Lehman 2015), a matter of concern with respect to the origin of an information-bearing genome.

AN EARLY RNA WORLD?

In all of the above scenarios, an autocatalytic mode of metabolism emerges before the appearance of any genetic machinery. Without a mode of inheritance or replication, such scenarios do not meet our definition of life, although they do offer some intriguing potential explanations for a number of cellular features, including the reliance on a proton-motive force for energy harvesting and the widespread deployment of transition metals in metabolic enzymes. The fact remains, however, that even if these suggested links to the past are correct, abiotically induced chemical reactions must have been eventually taken over completely by protein catalysts. And therein lies the problem. The spontaneous assembly of complex proteins is extraordinarily unlikely, and the refinement of catalytic properties in the absence

of a replicating genome is even less likely (if not impossible). However, all modern organisms are incapable of genome replication in the absence of a substantial set of helper proteins. This is the “chicken-and-egg” problem for the origin of life.

A potential solution to this problem is the idea that a single set of molecules jump-started life by serving simultaneously as catalysts and information-bearing polymers. Proteins carry out a bewildering diversity of tasks, but self-replication is not one of them. DNA provides a superb substrate for information storage, but the idea that DNA is catalytically inert led to the hypothesis that RNA is the only biomolecule for which some variants can both specify a genotype and express a phenotype, and therefore is the only reasonable candidate for a starting point in evolution. Invoking this default state, the RNA-world hypothesis postulates that at some point early in the evolution of life, before DNA had arrived on the scene, genetic continuity was assured by the replication of an RNA-based genome, with all underlying catalysis being carried out in the absence of proteins (Woese 1967; Crick 1968; Orgel 1968; Gilbert 1986; Robertson and Joyce 2012; Higgs and Lehman 2015). Under this view, the complex protein repertoire now employed by all organisms arose secondarily, layered on top of the more fundamental RNA scaffold.

Indirect support for this hypothesis derives from a number of observations. First, as with DNA, RNA’s use of four nucleotides constitutes a language, and the potential for double-strandedness allows for a template-based mechanism for replication. Second, with its ability to fold into complicated stem-loop structures, RNA is structurally diverse and capable of a wide variety of catalytic properties, including binding to proteins. Third, across of the Tree of Life, all of the major players in protein synthesis are derived from RNA – transfer RNAs, messenger RNAs, the catalytic cores of the ribosome and of the eukaryotic spliceosome, and numerous small RNAs involved in transcript silencing and/or proliferation. Fourth, many of the central players in metabolism are nucleotide derivatives, e.g., ATP, coenzyme A, NAD (nicotinamide adenine dinucleotide), and FAD (flavin adenine dinucleotide). Fifth, *in vitro* experiments on populations of RNA molecules demonstrate the evolvability of RNA in simple systems, having successfully selected for a wide variety of catalytic activities (e.g., Wilson and Szostak 1999; Joyce 2004).

If there was indeed an early RNA World, it may have coexisted with and even exploited one of the energy-generating scenarios outlined above, with successful members of the population of molecules gradually evolving to encode a mechanism for producing accessory proteins for enhancing the efficiency of energy harvesting, and eventually displacing the abiotic pathway entirely. Provided a population of such proto-genomes inhabited an environment spatially structured enough to ensure the association of metabolites with their source (e.g., a proto-membrane), such a setting might have initiated an auto-catalytic process of self-improvement on the path to what we now call life. Under this hypothesis, just as we see the proton-motive force as being an evolutionary relic, the diverse roles played by RNA in today’s cells, especially in transcript processing and translation, can be viewed as a relic of this early establishment of biochemistry.

There remains the fundamental question of how an information-bearing system can get started before the arrival of a system capable of exploiting information. One simple scenario is outlined in Foundations 2.2, where it is shown that provided there is a mechanism of polymerization and steady-state introduction of alternative

monomeric building blocks, an equilibrium population of polymers with variable lengths and contents will naturally evolve. Such a condition is expected to emerge even in the absence of a mechanism of self-replication, as the alternative states simply grow and decay out of a series of stochastic chemical reactions. Thus, it is not too far-fetched to imagine that a geochemical setting that provided a source of alternative ribonucleotides and a means for concatenating them would be primed towards developing a system of molecules carrying a potentially exploitable language.

Nonetheless, although it is often stated that the RNA-world hypothesis is widely accepted, a more appropriate statement might be that the RNA World is accepted as a hypothesis. One central caveat is that it is now known that like RNA, DNA (when maintained in single-stranded form, but allowed to fold into stems and loops) can take on a number of catalytic functions, including RNA cleavage, RNA and DNA ligation, and conjugation of amino acids to nucleotides (Silverman 2016).

Several other unresolved issues leave room for doubt about the extreme model in which RNA is the only replicator and the only catalyst (Shapiro 2006; McCollom 2013). First, there is the difficult question of how the basic building blocks of RNA, the ribonucleosides, were sustained and replenished. Mechanisms for the abiotic production of purine and pyrimidines have been demonstrated (Robertson and Joyce 2012; Becker et al. 2016), and ribose might also have arisen in the environments envisioned in the hydrothermal-vent and geothermal-field hypotheses, especially if boron was present (Kim et al. 2011; McCollom 2013; Neveu et al. 2013; Pearce et al. 2017). Under some conditions, most notably wet-dry cycles that may have occurred in geothermal fields, purines and pyrimidines can even be coupled with ribose to make nucleosides and further polymerized to small RNAs (Powner et al. 2009; Neveu et al. 2013; Becker et al. 2019). The speed of such reactions is not impressive, but early life had the luxury of time and lack of competition. Still, there remains the central problem that in today's organisms, nucleobases are synthesized from amino-acid precursors, the building blocks of protein, whose biosynthetic pathways are quite complex (Chapter 15).

Second, to jump-start the RNA World, there would not only need to be a pool of ribonucleotides (nucleobases joined with ribose), but these would need to be activated with pyrophosphates to promote chain growth. A mechanism to polymerize these basic building blocks would also be required, and to maintain continuity at least a subset of such polymers would have to be capable of self-replication (and of avoiding accidental replication of other competing genomes, which would stifle the process of natural selection).

Third, although some key steps towards self-replication have been accomplished (e.g., Lincoln and Joyce 2009), a fully self-replicating ribozyme has not yet been developed, despite considerable effort. Again, one could argue that the emergence of life had eons of time on an enormous planet in which to discover a few exceptional molecules, dwarfing the three decades of research performed in a handful of laboratories (the water in the oceans would fill $\sim 10^{23}$ 10-ml test tubes). Indeed, recent work in laboratory environments involving thermal gradients (Mast et al. 2013) or ice substrates (Attwater et al. 2013) have had success in developing ribozymes capable of polymerizing RNA sequences up to 200 bp in length, the approximate size generally necessary for catalytic activity.

Fourth, RNA is known to be maximally stable at slightly acidic pH (4.0 to 6.0)

and unstable in alkaline water. This reduces the appeal of the alkaline-vent hypothesis unless there was a spatial mechanism for decoupling metabolism and replication/information storage, but may be more compatible with the scenario postulated by the geothermal-field hypothesis (Bernhardt and Tate 2012).

Confronted with the special requirements for the assembly and maintenance of ribonucleotide polymers, some have suggested an RNA World preceded by a period dominated by some other polymer capable of genetic and catalytic functions (Joyce and Orgel 1999; Orgel 2000; Shapiro 2006). There is a wide variety of nucleotide analogs in which various moieties, including amino acids, are substituted for ribose (Robertson and Joyce 2012), and some of these are capable of nonenzymatic template copying (Pinheiro et al. 2012; Zhang et al. 2013). Why oligonucleotides at all? One argument is that such structures may have been chemically selected over other compounds, as they are powerful deactivators of UV light and exceptionally photostable. Thus, again assuming that life arose in an environment experiencing significant UV penetration, nucleobases may have served as protection of the pentose-phosphate backbones (Mulkidjanian et al. 2003; Serrano-Andrés and Merchán 2009; Dibrova et al. 2012).

The problem of the eventual transition to a DNA World remains. Two strong arguments favor DNA arising after both RNA and proteins. First, an early RNA-Protein World would imply the existence of a genetic code prior to the arrival of DNA, which is consistent with the ubiquitous use of transfer RNAs and ribosomal RNAs in translation. Second, modern cells derive their DNA building blocks (deoxyribonucleotides) from modifications of ribonucleotides. Ribonucleotide reductases are used in the production of dAMPs, dCMPs, and dGMPs, while thymidylate synthase produces dTMPs by methylating dUMP (Figure 2.5).

This additional step in dTMP synthesis suggests that the initial transition to a DNA World might have involved an intermediate form of DNA in which uracil was used instead of thymine. More remarkable are observations that the two primary prokaryotic lineages, the bacteria and the archaea (Chapter 3) utilize seemingly unrelated thymidylate synthases (Myllykallio et al. 2002) as well as two apparently unrelated sets of DNA-replication proteins (Edgell and Doolittle 1997; Olsen and Woese 1997; Leipe et al. 1999). Such observations raise the intriguing possibility that the shift to a DNA World may have occurred more than once.

Given the assumed early success of the RNA World, why would the transition to a DNA World be so complete as to eradicate all RNA-based genomes from the cellular domains of life? One attractive answer invokes two chemical features that reduce the mutational vulnerability of DNA-based genomes. First, the additional -OH group on ribose (Figure 2.5) renders RNA much less structurally stable than DNA. Second, one of the most common sources of mutation is cytosine deamination, which produces uracil. In thymine-bearing DNA, uracil can be recognized as aberrant and corrected prior to replication (assuming a mechanism for such recognition existed), but such a distinction is impossible in RNA. Thus, in an RNA World, an organism that discovered a way to store its genome as DNA while retaining RNA only for phenotypic purposes might have had a substantial advantage in terms of reliable genome propagation. Nonetheless, a transition between RNA and DNA Worlds would still have imposed three challenging requirements: a mechanism for the production of DNA nucleotides; a RNA-dependent DNA polymerase (i.e., a re-

verse transcriptase) for preserving a successful RNA sequence at the genomic level; and a DNA-dependent RNA-polymerase for the production of RNAs (Burton and Lehman 2009).

MEMBRANES AND THE EMERGENCE OF INDIVIDUALITY

Left unclear in the previous discussion is how a metabolism-first scenario, an RNA World, or a collaboration between the two might have lead to an eventual transition to an autonomous membrane-bound cell. Such encapsulation was almost certainly required before life could occupy the vast open space of marine environments, and without discrete individuals, the efficiency of natural selection would have been greatly diminished as any resources produced by a local entity would become public goods. On the negative side, cellularization reduces access to resources, although the earliest membranes might have been quite permeable.

Probably the most compelling reason to think that the last universal common ancestor was membrane-bound is the universal use of ATP synthase (noted above) as well as several other membrane-associated proteins (Jékely 2006; Mulikidjanian et al. 2009). All membranes in today's organisms consist of some form of lipid, and there are good reasons to think that this was the case in the earliest cells. First, although the concentrations are unknown, we can be virtually certain that lipids were present before the emergence of biology. Fatty acids have been found in extraterrestrial rocks such as the Murchison meteorite (Deamer 1985), and can be synthesized under plausible prebiotic conditions. Moreover, starting with CO, CO₂, and H₂ gases in the presence of metal catalysts at high temperature, a reaction known as Fischer-Tropsch synthesis can lead to long-chain fatty acids (McCollom et al. 1999). Second, given their amphiphilic nature, lipids spontaneously assemble into bilayered vesicles, with their hydrophobic tails forming the membrane interior and their hydrophilic heads pointing to the inside and outside of the vesicle (Chapter 15). Thus, it is quite plausible that lipids provided a natural starting point for membrane development even prior to the evolution of a genomically encoded mechanism for lipid biosynthesis.

The emergence of an autonomous cell would be much more likely if a genome, metabolism, and a membrane did not have to evolve independently in a stepwise fashion, but instead somehow facilitated each other's development. Some intriguing experiments demonstrate such possibilities. For example, Chen et al. (2004) developed a simple system containing model protocells made out of lipid bilayers, some empty and some containing RNA, showing that vesicles with high RNA concentrations experience osmotic stress that is relieved by the recruitment of lipid molecules from empty vesicles. This neatly demonstrates the intrinsic capacity of a genome-containing protocell to grow in the absence of any encoded mechanism for growth (and indeed in the absence of any initial genomic function at all). That is, if RNAs and lipids were colocalized in the same environment, the system would not only have been naturally biased towards spontaneous growth, but a sort of competition might have been set up, with the acquisition of membrane components being dominated by genome-containing vesicles. Once such a system was in place, any cell that contained a faster replicator would experience still more rapid growth, thereby

initiating a process of natural selection.

Following on earlier work by Walde et al. (1994), Mansy and Szostak (2009) took this sort of system even further. Starting with a setting containing DNA templates and DNA primers, they found that polymers could grow within a membrane-bound vesicle permeable to charged nucleotides, again causing osmotic pressure and vesicle growth. There remains, of course, the question of how membranes might come to be associated with nucleotides at all. Black et al. (2013) found that when colocalized, nucleotide bases and ribose not only associate with lipids, but stabilize the resultant aggregates in saline water. The fact that all of these features are a simple consequence of chemistry and physics again leads to the conclusion that rather than arising as a series of unimaginably low-probability events, molecular liaisons that constitute key aspects of life may have emerged semi-deterministically via natural abiotic processes.

Despite this progress, a number of gaps remain in our understanding of how membrane-bound life might have arisen. The unknowns include the mechanism by which repeated rounds of nucleotide polymerization might have been achieved, the avoidance of competition between strand reannealing and new chain growth, and the establishment of an organized mechanism of cell division. Prior to the emergence of a precise cell-division mechanism, protocell fission might have been governed simply by physical forces associated with the environment. For example, Zhu and Szostak (2009) found that as spherical vesicles grow, they eventually elongate into filamentous forms that are then subject to subdivision into daughter vesicles when the surrounding fluid is agitated. When associated with certain photochemically active compounds, filamentous vesicles can also differentiate into strings of ellipsoid subcompartments that eventually fragment into individual vesicles (Zhu et al. 2012). This phenomenon appears to result when photochemical induction of reactive oxygen species induces chemical changes that alter the surface tension of lipid membranes. From the observations on this simple system, one can imagine how a natural environment subject to redox cycles (perhaps driven by the light cycle) might have provided a purely physical mechanism for regulating protocell division.

Although membranes composed of short-chain fatty acids are highly permeable to a wide variety of nutrients, including amino acids and nucleotides, modern cells are generally bounded by phospholipid bilayers, which impose a much stronger barrier to charged ions and polar molecules. Assuming the earliest lipids were simple in form, how might a transition to phospholipids have come about? Budin and Szostak (2011) found that phospholipids can compete for incorporation into lipid membranes, raising the point that any cell that encoded a mechanism for producing such molecules from single-chain lipids might have gravitated toward the use of phospholipid membranes for purely physical reasons.

This, however, raises a secondary issue – the progressive establishment of a phospholipid membrane would bring with it reduced permeability and diminished access to the external environment. In principle, the gradual establishment of the phospholipid membrane may have fostered the emergence of internal cellular biosynthetic pathways to compensate for the reduction in external resource availability. However, any realistic scenario of this sort would have required a series of steps in which cellular fitness was never diminished. Natural selection allows populations to respond to selective challenges, but does not promote the latter.

Finally, it should be noted that all of the preceding views on the origin of membranes concerns are based on the assumption that the protoplasm of protocells resided inside cell membranes. A radical alternative view considers the opposite topology, with the protoplasm initially nucleating on the outside of vesicles called obcells, perhaps being held in place by actin-like filaments (Blobel 1980; Cavalier-Smith 2001; Griffiths 2007). In principle, such entities could have been cup-shaped, with the open side attached to a substrate (like a suction cup). Cellularization might then have evolved as the membrane of the liposome somehow completely invaginated (as in embryonic gastrulation) or as pairs of cups fused together, engulfing the previously external protoplasm.

GENOMIC CONSTRAINTS ON THE ESTABLISHMENT OF LIFE

Most origin-of-life researchers can be subdivided into two opposing camps. Those with a metabolism-first affinity find it unfathomable that an even moderately complex genome could ever be assembled prior to the establishment of a reliable source of energy for biocatalysis. In contrast, the genome-first school argues that complex catalytic pathways could never be assembled without the guiding hand of information-bearing molecules. Absent from most of this debate is the equally important matter of the maintenance of a population with heritable features capable of progressive adaptation.

Given its relative chemical stability, the arrival of DNA was a revolutionary event for evolution, providing a more permissive environment for genomic expansion and hence the emergence of more complex biological functions, but the power of natural selection is not limitless. To ensure a sustainable and productive path forward, an information-bearing molecule must be capable of generating accurate copies of itself. At the dawn of life, a level of replication fidelity much less accurate than in today's refined organisms would have imposed a significant restriction on genome size.

In the first attempt to grapple with this issue, Eigen (1971; Eigen and Schuster 1977) proposed the concept of a molecular quasispecies, with the master sequence being the genome with maximum fitness. Under this model, an error threshold is reached when the mutation rate is high enough that the master sequence cannot be maintained, i.e., the rate of promotion by selection is offset by mutational degradation to adjacent states. Loss of the master sequence need not imply extinction of the entire species, as suboptimal molecules may still have sufficient fitness to ensure numerical replacement of the population across generations. However, there exists a still higher mutation rate beyond which the population can no longer even be sustained (Bull et al. 2005). Theory from population genetics (Foundations 2.3) shows that for a given genome size, extinction avoidance requires a sufficiently low mutation rate that enough mutation-free offspring genomes are produced each generation to avoid permanent loss by stochastic sampling.

A key parameter dictating the fraction of deleterious mutation-free individuals in an asexual population is the ratio $\phi = U_d/s$, where U_d is the genome-wide deleterious mutation rate, and s is the fractional selective disadvantage of a deleterious mutation. The expected fraction of mutation-free individuals in a very large popu-

lation in selection-mutation balance is $e^{-\phi}$ (Foundations 2.3), so if ϕ is much larger than 1.0 (i.e., if the rate of introduction of deleterious mutations greatly exceeds the power of selection to remove them), mutation pressure alone will ensure the progressive loss of the highest fitness classes in all but enormous populations, eventually leading to population extinction by mutational meltdown. The generality of this principle underlies its application as a lethal-mutagenesis strategy for eradicating pathogens (Bull et al. 2005, 2007; Bull and Wilkie 2008; Chen and Shakhnovich 2009). For example, using laboratory populations of RNA molecules with a total error rate $> 10^{-5}$ per nucleotide site, Soll et al. (2007) showed that the time to extinction was positively related with population size, as theory predicts.

The concept of the mutational meltdown is based on the inevitable consequences of sampling of propagules from one generation to the next. Imagine that following a generation of selection the fraction of individuals in the best class is p , and that N random progeny are then derived from all surviving classes. The probability that a single draw does not contain a member of the best class is $(1 - p)$, and that of not drawing any members of this class at all is $(1 - p)^N$, where N is the current population size. If there is just a single individual in the best class ($p = 1/N$), then the probability of not drawing any progeny from this class is 0.367, and if there are two or three such individuals, the drops to 0.135 and 0.050, respectively. The main point is that if there are very few individuals in the best class, which will inevitably be the case with recurrent mutation pressure, there is an appreciable probability that the best class will be lost in any particular generation, and virtually certain probability that it will be lost over multiple generations. Once the best class is lost, the previously second-best class will be advanced to premier status, but it too will eventually suffer the same fate. With the mean fitness of individuals declining, a critical point will ultimately be reached when the average individual cannot replace itself, which causes a reduction in population size. This sets in motion a downward spiral towards extinction, as smaller population sizes increase the probability of loss of the best-class individuals, with each such loss leading to further loss in fitness.

The theory of the mutational meltdown (Foundations 2.3) is sufficiently well-developed that one can obtain mathematical approximations of the time to extinction given U_d and s . However, a more satisfying theory would start from first principles, generating an expected value of U_d resulting from selection for replication fidelity rather than simply assuming an arbitrary value. As outlined in Foundations 2.3, the strength of selection operating on the mutation rate in an asexual population is primarily a function of the difference in U_d among different genotypes, independent of the effects of individual mutations. Once selection has driven the mutation rate down to a sufficiently low level that the next increment of possible improvement in replication fidelity is smaller than the role of chance fluctuations in the population (resulting from random genetic drift; Chapter 5), no further improvement is possible. As a first-order approximation, for example, for a small population with 10^4 individuals, U_d can evolve to a level no lower than 10^{-4} . Because U_d is the product of the number of nucleotide sites in the genome and the mutation rate per site, for a population of any particular size, this puts an upper limit on the genome size consistent with maintaining sufficient numbers of mutation-free individuals to avoid the meltdown. Back and/or compensatory mutations may relieve the scenario outlined above somewhat (Wagner and Gabriel 1990; Poon and Otto 2000; Silander

et al. 2007; Goyal et al. 2012), but sufficiently high mutation pressure will still eventually lead to a mutational meltdown (Zeldovich et al. 2007; Chen and Shakhnovich 2009).

The relevance of these results is that levels of replication fidelity were likely quite low in the earliest stages of life. For example, polymerization off an RNA or RNA-like template in simple laboratory experiments typically yields error rates on the order of 0.01 to 0.1 per base incorporated even under optimal conditions (Johnston et al. 2001; Attwater et al. 2013; Zhang et al. 2013), many orders of magnitude higher than in any of today's cells. Because a genome with a length much greater than the inverse of the mutation rate per nucleotide site has essentially no chance of spawning an intact offspring molecule, this makes clear the challenge to early life – the need to encode for a high level of replication fidelity in an appropriately small genome. One possible way around this size-limitation problem is the joint operation of a set of suitably small cooperative molecules in a closed cycle with each member in the loop being responsible for the next member's replication (Attwater and Holliger 2012; Vaidya et al. 2012; Higgs and Lehman 2015). However, the salient point remains that a resolution of population-genetic issues is just as critical to understanding the origin of life as a focus on promising geological settings.

Summary

- Multiple lines of evidence demonstrate that several environments on the early Earth would have been conducive to the spontaneous emergence of most of the basic metabolic building blocks relied on in today's organisms, and may even explain the peculiar sets of reactions and metal cofactors upon which life came to depend.
- A wide range of observations indicate that the basic properties of life were set down ~ 3.5 to 4.0 billion years ago. However, the order in which the three major requirements for life – metabolism for resource acquisition, a genome for the heritable transmission of genetic information, and external membranes necessary for individuality – remains a matter of speculation.
- Given the core roles played by RNA in several key functions in today's organisms, the RNA World hypothesis provides a plausible means by which a single type of molecule could have simultaneously provided means for catalysis and information storage in the earliest stages of biotic evolution.
- One conceptual problem with origin-of-life narratives is that they are just that. As with the RNA World hypothesis, demonstrations of the plausibility of single steps towards life in restricted chemical/physical environments too often lead to increased adherence to narratives on the nature and order of events leading to the origin of life. This weaving of entire series of low-probability events into an

overall convincing scenario should be interpreted with caution.

- Putting aside the shortcomings in our understanding of the specific steps towards the establishment of the common ancestor of life, the experimental evidence suggests that, rather than being improbable, the origin of life may be a nearly inevitable consequence of the geochemical environments on early Earth-like planets.

Foundations 2.1. The proton-motive force and the evolution of ATP synthase. Life requires energy-capturing mechanisms to sustain the work necessary for cellular survival and reproduction. Although numerous sources of external energy are available to today's organisms, these must ultimately be converted to ATP, the universal currency of cellular energy storage and transport. Given that even a small bacterium requires the equivalent of about 30 billion ATP hydrolyses per cell division (Phillips and Milo 2009; Lynch and Marinov 2015; Chapter 8), the convoluted path by which ATP is produced and recycled is all the more remarkable.

Much like a dam generating electricity via water passing through a turbine, a process called “chemiosmosis” drives the cell's production of ATP by directing a gradient of hydrogen ions through channels in otherwise impermeable membranes (Figure 2.4). The protein complex involved, ATP synthase, sits in the cell membranes of prokaryotes and in the inner mitochondrial and chloroplast membranes of eukaryotes. However, unlike the situation with hydroelectric power, proton flow is not free. Instead, the proton gradient essential to the process is set up by the cell itself – using the electron-transport system, protons derived from the oxidation of food are translocated to the exterior of the membrane. They are then reimported through ATP synthase, where the energy associated with the proton-motive force is used to convert ADP and inorganic phosphate (usually denoted as Pi) into ATP. Although rare cases of substrate-level phosphorylation of ADP are known, almost all organisms rely chemiosmosis for regenerating ATP from ADP. Thus, we can be fairly certain that a membrane-embedded ATP synthase was used by the last (universal) common ancestor of all of life (LUCA).

Given the centrality of ATP synthase to bioenergetics, the establishment of this complex can be viewed as one of the key events in the history of cellular evolution. But why did life adopt such an arcane mechanism of energy harvesting? One possibility is that the reliance on a proton-motive force is a historical relic of the exploitable energy sources present at the time of life's foundation. Under this hypothesis, early life would have relied on an environmentally derived proton gradient (such as passive vent-associated energy) until establishing its own membrane-based mechanism for self-generating such a gradient and converting the mechanical energy from the returning proton movement to chemical energy in the form of ATP (Lane et al. 2010). If this view is correct, life could not have inhabited open-water environments (which do not provide strong, small-scale energy gradients) without first acquiring bioenergetic cell membranes and a sophisticated genome encoding them. Moreover, the implication is that the use of ATP as an energy carrier emerged prior to the evolution of ATP synthase.

ATP synthase typically consists of at least two dozen protein subunits (Figure 2.4), assembled into a membrane-bound pore (F_0), which in turn is connected to a central stalk that rotates within a large internal ring (F_1) kept stationary by a membrane-attached stator. Pushed by the pH gradient, protons flow through the complex, causing the stalk to rotate ~100 to 150 times/second (for comparison, the wheels of a car moving at 60 miles/hour rotate about 10× more slowly). Synthesis of ATP from ADP occurs as the rotating stalk interacts with the stationary F_1 ring carrying the catalytic subunits (Walker 2013).

Two types of ATP synthases are known: the so-called F-type found in bacteria and organelles, and the V-type found in archaea, some bacteria, and eukaryotic vesicle membranes (Cross and Müller 2004; Mulkidjanian et al. 2007, 2008). Owing to the complex structure of ATP synthase, the mechanisms by which its features evolved are far from clear. The probability of the sudden *de novo* evolutionary emergence of such a complex machine is minuscule, so the origin of ATP synthase most likely involved the exploitation of pre-existing modules engaged in other functions. The membrane subunit (F_0) could plausibly have been derived from a membrane pore (Walker 1998), and a relationship to the membrane-bound motor of the bacterial flagellum has also

been suggested (Mulikidjanian et al. 2007). Similarities also exist between the internal catalytic subunit (F_1) and the ring-like helicases that use energy released by ATP hydrolysis to separate the strands of DNA (Walker 1998; Patel and Picha 2000).

How any of these changes might have become established without the loss of ancestral gene functions remains unclear, but some involvement of gene duplication and reassignment seems almost certain (Chapter 5). Indeed, as will be further discussed below, the catalytic component of ATP synthase consists of a hexameric ring of two alternating subunits derived from an ancient gene duplication (with just one of the subunit types carrying out catalysis).

Despite the centrality of ATP synthase to energy harvesting, the enzyme exhibits significant structural variation with apparent functional implications. Bioenergetic efficiency is directly related to the structure of the membrane subunit (Soga et al. 2017). Each full rotation of the F_0 ring leads to the production of three ATP molecules (one for each of the subunits with catalytic properties), and the number of protons required per rotation is equal to the number of subunits in the rotating ring. Thus, in the yeast *Saccharomyces cerevisiae*, where there are ten subunits in the membrane ring, the bioenergetic cost is $10/3 = 3.33$ protons/ATP molecule produced. In bovine ATP synthase, however, there are only eight ring subunits, and sequence comparisons suggest that a similar structure may exist in all metazoans (Watt et al. 2010). For these species, the cost of each ATP is just $8/3 = 2.67$ protons. Across the Tree of Life, the number of ring subunits among characterized species ranges from 8 to 15, with known prokaryotic structures covering nearly the full range of variation (Walker 2013). Thus, in terms of proton utilization, there is a nearly twofold range of variation in the cost of ATP production among species. (A slight correction is that in mitochondria, there is an additional cost of one proton per ATP associated with the import of a phosphate group (Walker 2013), so the total cost of ATP production for eukaryotes is the above plus 1.0; this cost of membrane transport is not incurred in prokaryotes). Also not included in this analysis is the cost of producing the hydrogen-ion gradient itself.

Although these observations raise the question as to why all species don't utilize a system with the efficiency of that in the mammalian mitochondrion, this is not the only mystery posed by ATP synthase, as still other structural variants are known. For example, two key protein components normally present in the stator appear to be absent from parasitic apicomplexans and free-living ciliates, whereas the complex in the ciliate *Tetrahymena* contains at least thirteen novel proteins not found in other organisms, including the use of two stators rather than the one found in other characterized species (Balabaskaran Nina et al. 2010). In addition, members of the Chlamydomonadales (a group of green algae) have nine unique stator proteins (Lapaille et al. 2010), and *Euglena* ATP synthase has eight unique subunits (Mühleip et al. 2019). Many other differences are known in the bacteria and eukaryotes (Müller et al. 2005; Walker 2013).

If the preceding observations are not complicated enough, it should be noted that ATP synthases are actually reversible molecular machines that in certain contexts (e.g., vesicle acidification in eukaryotes) act as ATPases, with hydrogen ions being pumped with energy derived from the conversion of ATP to ADP. Moreover, a number of F- and V-type ATPases couple the synthesis/hydrolysis of ATP with the transport of sodium ions rather than protons, with some capable of using both. However, the interwoven phylogenetic relationships of the Na- and H-utilizing enzymes (Mulikidjanian et al. 2008) leave the ancestral state ambiguous and leave open the possibility of parallel evolution.

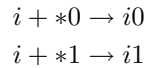
These scattered observations indicate that ATP synthase, a central requirement for all of life, has undergone substantial structural modifications despite the retention of a highly conserved function. There is, as yet, no evidence that any of these changes have been driven by adaptive processes, and many questions remain unanswered. What evolved first – the ability to synthesize ATP via a proton-motive force

(an ATP synthase), or the pumping of protons at the expense of ATP (an ATPase)? Why the use of a rotary motor? Why the reliance on ATP and not CTP or some other nucleoside triphosphate (or tetra- or higher-order phosphate)?

Foundations 2.2. Evolution prior to self-replication. Although ample evidence exists that most of the basic building blocks of life can emerge via abiotic processes, life requires the polymerization of monomeric subunits into linear arrays. How might populations of such polymers have initially come about in the absence of any mechanisms to specify their sequence or to exploit the information carried? And to what extent is the population of such molecules capable of evolution before a mechanism of replication has been acquired?

To examine these questions, Nowak and Ohtsuki (2008) considered a hypothetical prebiotic situation involving just two types of activated monomers, $*0$ and $*1$, each capable of joining a preexisting polymer on one side (like the joining of nucleotides only at the $3'$ end of a nucleic-acid chain). This type of model readily extends to situations with more than two monomers, but the general principles are most easily seen with just two alternative states at each site.

The possible polymeric states under this system consist of binary strings of various lengths: 0 and 1 for monomers; 00, 01, 10, and 11 for dimers; 000, 001, 010, 011, 100, 101, 110, and 111 for trimers, etc., so there are 2^L possible sequences of length L . Denoting an arbitrary polymer as i , there are two possible reactions



occurring at rates a_{i0} and a_{i1} (the absence of a $*$ indicates a recipient). The population of possible molecules can then be viewed as two nested trees (starting with either 0 or 1) (Figure 2.6). Each sequence i has a single possible precursor (i') and two possible descendants ($i0$ and $i1$).

Letting the rate of conversion of i' to i be a_i , assuming a death rate of i equal to d_i , and assuming a very large population size (so that stochastic fluctuations of frequencies can be ignored), a general description of the dynamics of the system is given by

$$\frac{dn_i}{dt} = a_i n_{i'} - (d_i + a_{i0} + a_{i1}) n_i, \quad (2.2.1)$$

where n_i is the abundance of string i . The first term on the right denotes the net flux from class i' into i , while the second term denotes the flux out of i resulting from either death (rate d_i) or the production of the next higher-order polymeric states (rate $a_{i0} + a_{i1}$). Assuming the monomeric precursors (0 and 1) are kept at a steady state arbitrarily scaled to $n_{0'} = n_{1'} = 1.0$, and letting $b_i = a_i / (d_i + a_{i0} + a_{i1})$, provided all $b_i > 0$, this system will eventually evolve to an equilibrium composition from any starting point,

$$n_i = b_i b_{i'} b_{i''} \cdots, \quad (2.2.2)$$

where the string of b coefficients goes back to the base of the tree (0 or 1). In words, the expected abundance of sequence i is simply equal to the product of all coefficients leading from its starting monomer. At this steady-state condition, for every string the total influx from the precursor is equal to total efflux into the two descendent string classes plus the death rate.

Although the general solution may be difficult to visualize, now imagine that both monomers behave identically, so that: 1) the baseline conversion rates from the

activated monomers *0 and *1 to states 0 and 1, respectively, are both equal to $\lambda/2$ (so the total rate of birth of polymerizable strings is equal to λ); 2) $a_i = a$ for all other classes (so all members of a particular length class grow at identical rates); and 3) there is a constant death rate per chain. At equilibrium, each of the 2^L possible sequences of length L then have abundance

$$n_L = \left(\frac{\lambda}{2a}\right) \left(\frac{a}{2a+d}\right)^L. \quad (2.2.3)$$

Because the fraction on the right is smaller than one, this shows that the abundance of sequences declines exponentially with the length, so that even though there is no physical upper limit imposed on L , long sequences become diminishingly rare. The mean sequence length is $1 + (2a/d)$, which is quite small unless $a \gg d$. Note, however, that long sequences are not rare because of any intrinsic fitness disadvantage, but simply because of the cumulative mortality of the $L - 1$ precursor sequences leading up to them. Scaled to the abundance of activated monomers (arbitrarily set equal to 1.0), the summed abundance over all strings (the total population size) is λ/d , i.e., the ratio of the rates of input and output for the population.

A number of key points arise from these results. First, although there is no self-replication in the system, a steady-state abundance distribution is maintained by the recurrent introduction of unit length strings (0 and 1). Second, if the rate constants take on different values, quite different distributions of string types will be obtained, i.e., the underlying conversion rates determine which classes the overall system becomes channeled into. In effect, with unequal transition rates, some classes will grow more rapidly than others, so that the system undergoes a kind of natural selection. Third, because the products of this birth-death process are polymers with alternative states at each site, the evolved system contains sequence diversity within each length class and hence potential information. Although this information is not actually utilized, such a system would be poised for exploitation once mechanisms of inheritance and replication were in place. Fourth, if some sequences do acquire the capacity for self-replication, there will be a critical rate of self-replication above which the behavior of the system can be radically altered, e.g., if only the largest molecules, which are normally kept rare by attrition, are capable of self-replication (Wu and Higgs 2009). More details on all of these matters and others can be found in Nowak and Ohtsuki (2008), Manapat et al. (2008, 2009), Ohtsuki and Nowak (2009), and Bianconi et al. (2013).

Foundations 2.3. The limits to genome replication fidelity. To understand the ultimate limits to any evolutionary phenomenon, we require theory to describe the average state of a population in the face of mutation, selection, and random genetic drift (where the latter is a result of stochasticity in inheritance in finite populations, as will be discussed in Chapter 5). Here, we apply some basic results from population-genetic theory to gain insight into the fundamental requirements for the establishment of life from the standpoint of genomic stability. Some of the results will be given without detailed explanations, which are postponed until subsequent chapters.

We assume an asexual population (with no exchange of genetic information among individuals) such that the genome of each offspring is a direct copy of that of its parent, barring mutation. The population is assumed to be of constant size (with N individuals), set by the level of resource availability and other ecological features. All mutations are assumed to be deleterious, and these to arise at rate U_d per genome per replication. Further assuming that each deleterious mutation independently reduces individual fitness by a fraction s (the selection coefficient), the fitness

of an individual with k mutations is

$$W_k = (1 - s)^k, \quad (2.3.1)$$

Under this model, the fitness of mutation-free ($k = 0$) individuals is equal to 1.0. This arbitrary baseline setting has no influence on the following results because selection operates via fitness differences relative to the population mean.

The relentless pressure from mutation will always result in a population with a breadth of fitness classes, with the relative abundances of the various classes being functions of the joint pressures of mutation towards higher k and selection favoring smaller k (Haigh 1978). A central question is whether the number of individuals in the $k = 0$ class is sufficiently large to avoid loss by stochastic sampling across generations.

Muller (1964) first pointed out that unless s is large enough that mutations are eliminated rapidly, only a small minority of individuals (if any) typically occupy the $k = 0$ class. Unless this number, N_0 , is sufficiently large, the best class will eventually be lost, as there will eventually be a chance generation in which no member of the best class leaves mutation-free progeny. At that point, the second-best class will be elevated to superior status, but it too will eventually suffer the fate of being replaced by the third best class, and so on. This phenomenon, which leads to a progressive loss of fitness, was called Muller's ratchet by Felsenstein (1974), and numerous authors have attempted to solve the difficult problem of the rate at which the ratchet clicks (e.g., Gabriel et al. 1993; Stephan et al. 1993; Gessler 1995; Gordo and Charlesworth 2000). Once initiated, Muller's ratchet can eventually lead to the point at which a population can no longer replace itself. This then leads to an accelerating approach to extinction via a mutational meltdown, whereby progressive declines in population size encourage still faster stochastic clicks of the ratchet (Lynch and Gabriel 1990; Lynch et al. 1993).

The key determinant of whether a population can avoid the ratchet is the expected number of individuals in the $k = 0$ class. For very large N , the frequency distribution of the numbers of individuals in the various classes is Poisson, with an average number of deleterious mutations per individual of U_d/s (Haigh 1978). The form of a Poisson distribution is a simple function of the mean, with the expected number of individuals in the lowest ($k = 0$) class being

$$N_0 = N e^{-U_d/s}, \quad (2.3.2)$$

where N is the total population size. This number is critical to a population's ability to withstand mutation pressure. The remaining $N - N_0$ mutation-carrying individuals are effectively the "living dead," as all future descendants of the population must ultimately trace back to the $k = 0$ class if the population is to avoid descent down the path of mutational degradation.

We now consider two fundamental issues: 1) the critical genome size above which U_d is so high that Muller's ratchet will rapidly proceed; and 2) the degree to which natural selection to reduce the mutation rate can ameliorate this process. If the ratchet is to be stopped, the power of selection against new mutations arising in the $k = 0$ subclass must substantially exceed the random fluctuations in allele frequencies caused by drift, the variance of which is proportional to the inverse of the sample size (Chapter 5). In this case, we are concerned with fluctuations in the best class, so avoidance of the ratchet requires $s \gg 1/N_0$, or equivalently $sN_0 \gg 1$. Substituting for N_0 from Equation 2.3.2 and rearranging, the critical genomic mutation rate below which the ratchet effectively stops is found to be

$$U_d \ll s \ln(sN). \quad (2.3.3a)$$

Now note that for a genome size of n nucleotides, U_d can be expressed as nu_d , where u_d is the deleterious mutation rate per nucleotide site. Thus, Equation (2.3.3a)

implies an upper limit to a sustainable genome size of

$$n_s \simeq [s \ln(sN)]/u_d. \quad (2.3.3b)$$

Strictly speaking, n_s refers only to genomic sites at which a nucleotide substitution has fitness consequences. For example, RNA molecules with catalytic properties typically assemble into complex structures containing stems (consisting of complementary base pairs) and loops, with the loop sequences often being of negligible importance so long as the loop is retained. Thus, the total sustainable genome size in an RNA-world organism could have exceeded n_s to the extent that effectively neutral sites were present in the genome (Kun et al. 2005).

The preceding derivation makes clear that with a lower mutation rate, there is more room for genome-size expansion, but leaves unexplained the evolution of the mutation rate itself. To achieve such an understanding, we require an expression for the selective advantage of high replication fidelity. In an asexual species, such selection operates through the deleterious mutation loads that become trapped in lineages with different mutation rates. Letting ΔU_d denote the difference in deleterious mutation rates between two lineages, and recalling that the selection coefficient s is the measure of the rate of removal of individuals with an excess mutation, the excess equilibrium mean number of mutations in the lineage with the higher mutation rate is the ratio of the elevated rate of input to the rate of removal by selection, $\Delta U_d/s$. The fitness difference among genotypes is the product of this excess number and the reduction in fitness per mutation, $(\Delta U_d/s) \times s = \Delta U_d$. Thus, the selective disadvantage of a genotype with an elevated mutation rate is simply equal to the increase in the genome-wide deleterious mutation rate, independent of the effects of individual mutations (Kimura 1967; Johnson 1999a; Lynch 2008).

This result allows a simple statement on the degree to which selection can reduce U_d . As just noted, selection is ineffective unless its magnitude exceeds the power of genetic drift. Because the maximum possible selective disadvantage of a hypothetical genotype is obtained by contrasting with the expectations for a genotype with perfect replication fidelity ($U_d = 0$), the absolute lower limit to the evolvable genome-wide deleterious mutation rate is on the order of

$$U_d^* = 1/N. \quad (2.3.4)$$

This follows because the power of drift can be no smaller than $1/N$ (Lynch 2010, 2011), and from the above-point that selection is ineffective if its magnitude is lower than the diffusive power of drift.

Thus, Equation 2.3.4 tells us that selection is unable to drive U_d below $1/N$, whereas Equation 2.3.3a tells us $U_d > s \ln(sN)$ is inconsistent with sustainable life in the absence of recombination. It then follows that there must be a critical population size (N^*) below which selection is incapable of driving U_d to a low enough level to avoid eventual extermination by a mutational meltdown. The solution, obtained by equating Equations 2.3.3a and 2.3.4,

$$(sN^*) \ln(sN^*) = 1,$$

which simplifies to

$$N^* = 1.76/s. \quad (2.3.5)$$

Notice that this critical population size is independent of the mutation rate, depending only on the average effect of deleterious mutations. It is difficult to say what the magnitude of s might have been at the early stages of life, although in modern-day species the average value of s is on the order of 0.001 to 0.01 (Lynch et

al. 1999). Thus, Equation 2.3.5 implies that the absolute minimum population size critical to the establishment of a stable primordial life form is a few hundred to a few thousand genomes.

Aside from the potentially serious problem of simple population loss by physical accidents, there are a number of reasons why this number is certainly an underestimate (perhaps by orders of magnitude). First, the drift barrier to mutation-rate evolution of $1/N$ is strictly valid only if mutations influencing the mutation rate are equally distributed in the upward and downward directions. The critical population size would be elevated if mutator alleles arise more frequently than antimutators (Lynch et al. 2016). Second, Equation 2.3.4 is derived by drawing a contrast with the extreme case of a genome with perfect replication fidelity. In reality, the contrast should be made between adjacent possible changes on the scale of replication fidelity, i.e., between the current and next best rate, which will be much smaller.

The previous results rely on the assumption that all mutations are deleterious. Attempts have been made to define the optimal mutation rate for maximizing the long-term rate of adaptive evolution when unconditionally beneficial and deleterious mutations are occurring simultaneously (Leigh 1970, 1973; Orr 2000; Bull 2008; Gerrish et al. 2013), but because selection operates on the immediate time scale (rather than looking to the future), it is unclear whether such rates are ever achievable (Sturtevant 1937; Johnson 1999b; Clune et al. 2008; Desai and Fisher 2011). Occasionally, a mutator allele may be brought to high-frequency by hitch-hiking with a tightly linked beneficial mutation, as in cases of mismatch-repair deficient pathogens acquiring antibiotic resistance (LeClerc et al. 1996; Denamur and Matic 2006; Giraud et al. 2007). However, such events are generally transient, as they are quickly followed by reversion of the mutation rate (André and Godelle 2006; Gerrish et al. 2007; Raynes et al. 2012). Thus, taken together, theory and empirical observations (Chapter 4) lead to the conclusion that selection primarily drives mutation rates in a downward direction, with an ultimate barrier to what can be achieved being set by the size of the population (Lynch 2011; Lynch et al. 2016).

Literature Cited

- André, J. B., and B. Godelle. 2006. The evolution of mutation rate in finite asexual populations. *Genetics* 172: 611-626.
- Attwater, J., and P. Holliger. 2012. Origins of life: the cooperative gene. *Nature* 491: 48-49.
- Attwater, J., A. Wochner, and P. Holliger. 2013. In-ice evolution of RNA polymerase ribozyme activity. *Nature Chem.* 5: 1011-1018.
- Balabaskaran Nina, P., N. V. Dudkina, L. A. Kane, J. E. van Eyk, E. J. Boekema, M. W. Mather, and A. B. Vaidya. 2010. Highly divergent mitochondrial ATP synthase complexes in *Tetrahymena thermophila*. *PLoS Biol.* 8: e1000418.
- Baross, J. A. and S. E. Hoffman. 1985. Submarine hydrothermal vents and associated gradient environments as sites for the origin and evolution of life. *Origins of Life* 15: 327-345.
- Becker, S., I. Thoma, A. Deutsch, T. Gehrke, P. Mayer, H. Zipse, and T. Carell. 2016. A high-yielding, strictly regioselective prebiotic purine nucleoside formation pathway. *Science* 352: 833-836.
- Becker, S., J. Feldmann, S. Wiedemann, H. Okamura, C. Schneider, K. Iwan, A. Crisp, M. Rossa, T. Amatov, and T. Carell. 2019. Unified prebiotically plausible synthesis of pyrimidine and purine RNA ribonucleotides. *Science* 366: 76-82.
- Bell, E. A., P. Boehnke, T. M. Harrison, and W. L. Mao. 2015. Potentially biogenic carbon preserved in a 4.1 billion-year-old zircon. *Proc. Natl. Acad. Sci. USA* 112: 14518-14521.
- Benner, S. A., H. J. Kim, M. J. Kim, and A. Ricardo. 2010. Planetary organic chemistry and the origins of biomolecules. *Cold Spring Harbor Perspect. Biol.* 2: a003467.
- Bernhardt, H. S., and W. P. Tate. 2012. Primordial soup or vinaigrette: did the RNA world evolve at acidic pH? *Biol. Direct* 7: 4.
- Bianconi, G., K. Zhao, I. A. Chen, and M. A. Nowak. 2013. Selection for replicases in protocells. *PLoS Comput. Biol.* 9: e1003051.
- Black, R. A., M. C. Blosser, B. L. Stottrup, R. Tavakley, D. W. Deamer, and S. L. Keller. 2013. Nucleobases bind to and stabilize aggregates of a prebiotic amphiphile, providing a viable mechanism for the emergence of protocells. *Proc. Natl. Acad. Sci. USA* 110: 13272-13276.
- Blobel, G. 1980. Intracellular protein topogenesis. *Proc. Natl. Acad. Sci. USA* 77: 1496-1500.
- Bottke, W. F., D. Vokrouhlický, D. Minton, D. Nesvorný, A. Morbidelli, R. Brasser, B. Simonson, and H. F. Levison. 2012. An Archaean heavy bombardment from a destabilized extension of the asteroid belt. *Nature* 485: 78-81.
- Boyer, P. D. 2002. A research journey with ATP synthase. *J. Biol. Chem.* 277: 39045-39061.
- Brasier, M. D., J. Antcliffe, M. Saunders, and D. Wacey. 2015. Changing the picture of Earth's earliest fossils (3.5-1.9 Ga) with new approaches and new discoveries. *Proc. Natl. Acad. Sci. USA* 112: 4859-4864.
- Budin, I., and J. W. Szostak. 2011. Physical effects underlying the transition from primitive to modern cell membranes. *Proc. Natl. Acad. Sci. USA* 108: 5249-5254.
- Bull J. J. 2008. The optimal burst of mutation to create a phenotype. *J. Theor. Biol.* 254: 667-673.

- Bull J. J., L. A. Meyers, and M. Lachmann. 2005. Quasispecies made simple. *PLoS Comput. Biol.* 1: e61.
- Bull, J. J., R. Sanjuán, and C. O. Wilke. 2007. Theory of lethal mutagenesis for viruses. *J. Virol.* 81: 2930-2939.
- Bull, J. J., and C. O. Wilke. 2008. Lethal mutagenesis of bacteria. *Genetics* 180: 1061-1070.
- Burton, A. S., and N. Lehman. 2009. DNA before proteins? Recent discoveries in nucleic acid catalysis strengthen the case. *Astrobiol.* 9: 125-130.
- Cammack, R., K. K. Rao, and D. O. Hall. 1981. Metalloproteins in the evolution of photosynthesis. *Biosystems* 14: 57-80.
- Cavalier-Smith, T. 2001. Obcells as proto-organisms: membrane heredity, lithophosphorylation, and the origins of the genetic code, the first cells, and photosynthesis. *J. Mol. Evol.* 53: 555-595.
- Chen, I. A., R. W. Roberts, and J. W. Szostak. 2004. The emergence of competition between model protocells. *Science* 305: 1474-1476.
- Chen, P., and E. I. Shakhnovich. 2009. Lethal mutagenesis in viruses and bacteria. *Genetics* 183: 639-650.
- Clune, J., D. Misevic, C. Ofria, R. E. Lenski, S. F. Elena, and R. Sanjuán. 2008. Natural selection fails to optimize mutation rates for long-term adaptation on rugged fitness landscapes. *PLoS Comput. Biol.* 4: e1000187.
- Cnossen, I., J. Sanz-Forcada, F. Favata, O. Witasse, T. Zegers, and N. F. Arnold. 2007. Habitat of early life: solar X-ray and UV radiation at Earth's surface 4-3.5 billion years ago. *J. Geophys. Res.* 112: E02008.
- Cody, G. D. 2004. Transition metal sulfides and the origins of metabolism. *Annu. Rev. Earth Planet. Sci.* 32: 569-599.
- Cody, G. D., N. Z. Boctor, T. R. Filley, R. M. Hazen, J. H. Scott, A. Sharma, and H. S. Yoder, Jr. 2000. Primordial carbonylated iron-sulfur compounds and the synthesis of pyruvate. *Science* 289: 1337-1340.
- Cooper, G., C. Reed, D. Nguyen, M. Carter, and Y. Wang. 2011. Detection and formation scenario of citric acid, pyruvic acid, and other possible metabolism precursors in carbonaceous meteorites. *Proc. Natl. Acad. Sci. USA* 108: 14015-14020.
- Corliss, J. B., J. A. Baross, and S. E. Hoffman. 1981. An hypothesis concerning the relationships between submarine hot springs and the origin of life on Earth. *Oceanologica Acta* 4: 59-69.
- Crick, F. H. 1968. The origin of the genetic code. *J. Mol. Biol.* 38: 367-379.
- Cross, R. L., and V. Müller. 2004. The evolution of A-, F-, and V-type ATP synthases and ATPases: reversals in function and changes in the H⁺/ATP coupling ratio. *FEBS Lett.* 576: 1-4.
- Damer, B., and D. Deamer. 2015. Coupled phases and combinatorial selection in fluctuating hydrothermal pools: a scenario to guide experimental approaches to the origin of cellular life. *Life (Basel)* 5: 872-887.
- David, L. A., and E. J. Alm. 2011. Rapid evolutionary innovation during an Archaean genetic

- expansion. *Nature* 469: 93-96.
- Deamer, D. W. 1985. Boundary structures are formed by organic components of the Murchison carbonaceous chondrites. *Nature* 317: 792-794.
- Deamer, D. W., and C. D. Georgiou. 2015. Hydrothermal conditions and the origin of cellular life. *Astrobiology* 15: 1091-1095.
- DeDuve, C. 1991. *Blueprint for a Cell*. Neil Patterson Publ., Burlington, NC.
- Denamur, E., and I. Matic. 2006. Evolution of mutation rates in bacteria. *Mol. Microbiol.* 60: 820-827.
- Desai, M. M., and D. S. Fisher. 2011. The balance between mutators and nonmutators in asexual populations. *Genetics* 188: 997-1014.
- Dibrova, D. V., M. Y. Chudetsky, M. Y. Galperin, E. V. Koonin, and A. Y. Mulkidjanian. 2012. The role of energy in the emergence of biology from chemistry. *Orig. Life Evol. Biosph.* 42: 459-468.
- Dupont, C. L., A. Butcher, R. E. Valas, P. E. Bourne, and G. Caetano-Anollés G. 2010. History of biological metal utilization inferred through phylogenomic analysis of protein structures. *Proc. Natl. Acad. Sci. USA* 107: 10567-10572.
- Edgell, D. R., and W. F. Doolittle. 1997. Archaea and the origin(s) of DNA replication proteins. *Cell* 89: 995-998.
- Eigen, M. 1971. Selforganization of matter and the evolution of biological macromolecules. *Naturwissenschaften* 58: 465-523.
- Eigen, M., and P. Schuster. 1977. The hypercycle. A principle of natural self-organization. Part A: Emergence of the hypercycle. *Naturwissenschaften* 64: 541-565.
- Felsenstein, J. 1974. The evolutionary advantage of recombination. *Genetics* 78: 737-756.
- Felsenstein, J. 2004. *Inferring Phylogenies*. Sinauer Assocs., Inc. Sunderland, MA.
- Furnes, H., N. R. Banerjee, K. Muehlenbachs, H. Staudigel, and M. de Wit. 2004. Early life recorded in archean pillow lavas. *Science* 304: 578-581.
- Gabriel, W., M. Lynch, and R. Bürger. 1993. Muller's ratchet and mutational meltdowns. *Evolution* 47: 1744-1757.
- Gerrish, P. J., A. Colato, A. S. Perelson, and P. D. Sniegowski. 2007. Complete genetic linkage can subvert natural selection. *Proc. Natl. Acad. Sci. USA* 104: 6266-6271.
- Gerrish, P. J., A. Colato, and P. D. Sniegowski. 2013. Genomic mutation rates that neutralize adaptive evolution and natural selection. *J. R. Soc. Interface* 10: 20130329.
- Gessler, D. D. 1995. The constraints of finite size in asexual populations and the rate of the ratchet. *Genet. Res.* 66: 241-253.
- Gilbert, W. 1986. The RNA world. *Nature* 319: 618.
- Giraud, A., M. Radman, I. Matic, and F. Taddei. 2001. The rise and fall of mutator bacteria. *Curr. Opin. Microbiol.* 4: 582-585.
- Goldford, J. E., H. Hartman, T. F. Smith, and D. Segré. 2017. Remnants of an ancient metabolism without phosphate. *Cell* 168: 1126-1134.

- Gordo, I., and B. Charlesworth. 2000. The degeneration of asexual haploid populations and the speed of Muller's ratchet. *Genetics* 154: 1379-1387.
- Goyal, S., D. J. Balick, E. R. Jerison, R. A. Neher, B. I. Shraiman, and M. M. Desai. 2012. Dynamic mutation-selection balance as an evolutionary attractor. *Genetics* 191: 1309-1319.
- Griffiths, G. 2007. Cell evolution and the problem of membrane topology. *Nat. Rev. Mol. Cell Biol.* 8: 1018-1024.
- Haigh, J. 1978. The accumulation of deleterious genes in a population – Muller's ratchet. *Theor. Popul. Biol.* 14: 251-267.
- Haldane, J. B. S. 1929. The origin of life. *Rationalist Annual* 3: 148-169.
- Hall, D. O., R. Cammack, and K. K. Rao. 1974. The iron-sulphur proteins: evolution of a ubiquitous protein from model systems to higher organisms. *Orig. Life* 5: 363-386.
- Hazen, R. M., and D. W. Deamer. 2007. Hydrothermal reactions of pyruvic acid: synthesis, selection, and self-assembly of amphiphilic molecules. *Orig. Life Evol. Biosph.* 37: 143-152.
- Higgs, P. G., and N. Lehman. 2015. The RNA World: molecular cooperation at the origins of life. *Nat. Rev. Genet.* 16: 7-17.
- Jékely, G. 2006. Did the last common ancestor have a biological membrane? *Biol. Direct* 1: 35.
- Johnson, A. P., H. J. Cleaves, J. P. Dworkin, D. P. Glavin, A. Lazcano, and J. L. Bada. 2008. The Miller volcanic spark discharge experiment. *Science* 322: 404.
- Johnson, B. C., and H. J. Melosh. 2012. Impact spherules as a record of an ancient heavy bombardment of Earth. *Nature* 485: 75-77.
- Johnson, T. 1999a. The approach to mutation-selection balance in an infinite asexual population, and the evolution of mutation rates. *Proc. R. Soc. Lond. B* 266: 2389-2397.
- Johnson, T. 1999b. Beneficial mutations, hitchhiking and the evolution of mutation rates in sexual populations. *Genetics* 151: 1621-1631.
- Johnston, W. K., P. J. Unrau, M. S. Lawrence, M. E. Glasner, and D. P. Bartel. 2001. RNA-catalyzed RNA polymerization: accurate and general RNA-templated primer extension. *Science* 292: 1319-1325.
- Joyce, G. F. 2004. Directed evolution of nucleic acid enzymes. *Ann. Rev. Biochem.* 73: 791-836.
- Joyce, G. F., and L. E. Orgel. 1999. Prospects for understanding the origin of the RNA World, pp. 49-77. In R. F. Gesteland, T. R. Cech, and J. F. Atkins (eds.) *The RNA World*, 2nd Ed. Cold Spring Harbor Lab. Press, Cold Spring Harbor, NY.
- Keller, M. A., A. V. Turchyn, and M. Ralser. 2014. Non-enzymatic glycolysis and pentose phosphate pathway-like reactions in a plausible Archean ocean. *Mol. Syst. Biol.* 10: 725.
- Kim, H. J., A. Ricardo, H. I. Illangkoon, M. J. Kim, M. A. Carrigan, F. Frye, and S. A. Benner. 2011. Synthesis of carbohydrates in mineral-guided prebiotic cycles. *J. Am. Chem. Soc.* 133: 9457-9468.
- Kimura, M. 1967. On the evolutionary adjustment of spontaneous mutation rates. *Genet. Res.* 9: 23-34.
- Knoll, A. H. 2004. *Life on a Young Planet: the First Three Billion Years of Evolution on Earth*. Princeton University Press, Princeton, NJ.

- Kun, A., M. Santos, and E. Szathmáry. 2005. Real ribozymes suggest a relaxed error threshold. *Nat. Genet.* 37: 1008-1011.
- Lane, N., J. F. Allen, and W. Martin. 2010. How did LUCA make a living? Chemiosmosis in the origin of life. *Bioessays* 32: 271-280.
- Lane, N., and W. F. Martin. 2012. The origin of membrane bioenergetics. *Cell* 151: 1406-1416.
- Lapaille, M., A. Escobar-Ramírez, H. Degand, D. Baurain, E. Rodríguez-Salinas, N. Coosemans, M. Boutry, D. Gonzalez-Halphen, C. Remacle, and P. Cardol. 2010. Atypical subunit composition of the chlorophycean mitochondrial F_1F_0 -ATP synthase and role of Asa7 protein in stability and oligomycin resistance of the enzyme. *Mol. Biol. Evol.* 27: 1630-1644.
- LeClerc, J. E., B. Li, W. L. Payne, and T. A. Cebula. 1996. High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. *Science* 274: 1208-1211.
- Leigh, E. G., Jr. 1970. Natural selection and mutability. *Amer. Natur.* 104: 301-305.
- Leigh, E. G., Jr. 1973. The evolution of mutation rates. *Genetics (Suppl.)* 73: 1-18.
- Leipe, D. D., L. Aravind, and E. V. Koonin. 1999. Did DNA replication evolve twice independently? *Nucleic Acids Res.* 27: 3389-3401.
- Lincoln, T. A., and G. F. Joyce. 2009. Self-sustained replication of an RNA enzyme. *Science* 323: 1229-1232.
- Lynch, M. 2008. The cellular, developmental, and population-genetic determinants of mutation-rate evolution. *Genetics* 180: 933-943.
- Lynch, M. 2010. Evolution of the mutation rate. *Trends Genet.* 26: 345-352.
- Lynch, M. 2011. The lower bound to the evolution of mutation rates. *Genome Biol. Evol.* 3: 1107-1118.
- Lynch, M., M. Ackerman, J.-F. Gout, H. Long, W. Sung, W. K. Thomas, and P. L. Foster. 2016. Genetic drift, selection, and evolution of the mutation rate. *Nat. Rev. Genetics* 17: 704-714.
- Lynch, M., J. Blanchard, D. Houle, T. Kibota, S. Schultz, L. Vassilieva, and J. Willis. 1999. Spontaneous deleterious mutation. *Evolution* 53: 645-663.
- Lynch, R. Bürger, D. Butcher, and W. Gabriel. 1993. Mutational meltdowns in asexual populations. *J. Heredity* 84: 339-344.
- Lynch, M., and W. Gabriel. 1990. Mutation load and the survival of small populations. *Evolution* 44: 1725-1737.
- Lynch, M., and G. K. Marinov. 2015. The bioenergetic costs of a gene. *Proc. Natl. Acad. Sci. USA* 112: 15690-15695.
- Maden, B. E. 1995. No soup for starters? Autotrophy and the origins of metabolism. *Trends Biochem. Sci.* 20: 337-341.
- Manapat, M. L., I. A. Chen, and M. A. Nowak. 2010. The basic reproductive ratio of life. *J. Theor. Biol.* 263: 317-327.
- Manapat, M., H. Ohtsuki, R. Bürger, and M. A. Nowak. 2009. Originator dynamics. *J. Theor. Biol.* 256: 586-595.
- Mansy, S. S., and J. W. Szostak. 2009. Reconstructing the emergence of cellular life through the

- synthesis of model protocells. *Cold Spring Harb. Symp. Quant. Biol.* 74: 47-54.
- Martin, W., J. Baross, D. Kelley, and M. J. Russell. 2008. Hydrothermal vents and the origin of life. *Nat. Rev. Microbiol.* 6: 805-814.
- Martin, W., and M. J. Russell. 2003. On the origins of cells: a hypothesis for the evolutionary transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from prokaryotes to nucleated cells. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 358: 59-83.
- Mast, C. B., S. Schink, U. Gerland, and D. Braun. 2013. Escalation of polymerization in a thermal gradient. *Proc. Natl. Acad. Sci. USA* 110: 8030-8035.
- McCollom, T. M. 2013. Miller-Urey and beyond: what have we learned about prebiotic organic synthesis reactions in the past 60 years? *Ann. Rev. Earth Planet. Sci.* 41: 207-229.
- McCollom, T. M., G. Ritter, and B. R. T. Simoneit. 1999. Lipid synthesis under hydrothermal conditions by Fischer-Tropsch-Type reactions. *Origins Life Evol. Biosphere* 29: 153-166.
- Miller, S. L. 1953. Production of amino acids under possible primitive earth conditions. *Science* 117: 528-529.
- Miller, S. L., and H. C. Urey. 1959. Organic compound synthesis on the primitive Earth. *Science* 130: 245-251.
- Muchowska, K. B., S. J. Varma, and J. Moran. 2019. Synthesis and breakdown of universal metabolic precursors promoted by iron. *Nature* 569: 104-107.
- Mühleip, A., S. E. McComas, and A. Amunts. 2019. Structure of a mitochondrial ATP synthase with bound native cardiolipin. *Elife* pii: e51179.
- Mulkidjanian, A. Y. 2009. On the origin of life in the zinc world: 1. Photosynthesizing, porous edifices built of hydrothermally precipitated zinc sulfide as cradles of life on Earth. *Biol. Direct* 4: 26.
- Mulkidjanian, A. Y., A. Y. Bychkov, D. V. Dibrova, M. Y. Galperin, and E. V. Koonin. 2012a. Open questions on the origin of life at anoxic geothermal fields. *Orig. Life Evol. Biosph.* 42: 507-516.
- Mulkidjanian, A. Y., A. Y. Bychkov, D. V. Dibrova, M. Y. Galperin, and E. V. Koonin. 2012b. Origin of first cells at terrestrial, anoxic geothermal fields. *Proc. Natl. Acad. Sci. USA* 109: E821-E830.
- Mulkidjanian, A. Y., D. A. Cherepanov, and M. Y. Galperin. 2003. Survival of the fittest before the beginning of life: selection of the first oligonucleotide-like polymers by UV light. *BMC Evol. Biol.* 3: 12.
- Mulkidjanian, A. Y., and M. Y. Galperin. 2009. On the origin of life in the zinc world. 2. Validation of the hypothesis on the photosynthesizing zinc sulfide edifices as cradles of life on Earth. *Biol. Direct* 4: 27.
- Mulkidjanian, A. Y., M. Y. Galperin, K. S. Makarova, Y. I. Wolf, and E. V. Koonin. 2008. Evolutionary primacy of sodium bioenergetics. *Biol. Direct* 3: 13.
- Mulkidjanian, A. Y., M. Y. Galperin, and E. V. Koonin. 2009. Co-evolution of primordial membranes and membrane proteins. *Trends Biochem. Sci.* 34: 206-215.
- Mulkidjanian, A. Y., K. S. Makarova, M. Y. Galperin, and E. V. Koonin. 2007. Inventing the

- dynamo machine: the evolution of the F-type and V-type ATPases. *Nat. Rev. Microbiol.* 5: 892-899.
- Muller, H. J. 1964. The relation of recombination to mutational advance. *Mutation Res.* 1: 2-9.
- Müller, V., A. Lingl, K. Lewalter, and M. Fritz. 2005. ATP synthases with novel rotor subunits: new insights into structure, function and evolution of ATPases. *J. Bioenerg. Biomembr.* 37: 455-460.
- Myllykallio, H., G. Lipowski, D. Leduc, J. Filée, P. Forterre, and U. Liebl. 2002. An alternative flavin-dependent mechanism for thymidylate synthesis. *Science* 297: 105-107.
- Neveu, M., H. J. Kim, and S. A. Benner. 2013. The “strong” RNA world hypothesis: fifty years old. *Astrobiol.* 13: 391-403.
- Nisbet, E. G., and N. H. Sleep. 2001. The habitat and nature of early life. *Nature* 409: 1083-1091.
- Novikov, Y., and S. D. Copley. 2013. Reactivity landscape of pyruvate under simulated hydrothermal vent conditions. *Proc. Natl. Acad. Sci. USA* 110: 13283-13288.
- Nowak, M. A., and H. Ohtsuki. 2008. Prevolutionary dynamics and the origin of evolution. *Proc. Natl. Acad. Sci. USA* 105: 14924-14927.
- Ohtsuki, H., and M. A. Nowak. 2009. Pre-life catalysts and replicators. *Proc. R. Soc. B* 276: 3783-3790.
- Olsen, G. J., and C. R. Woese. 1997. Archaeal genomics: an overview. *Cell* 89: 991-994.
- Oparin, A. I. 1938. *Origin of Life*. Macmillan Co., New York, NY.
- Orgel, L. E. 1968. Evolution of the genetic apparatus. *J. Mol. Biol.* 38: 381-393.
- Orgel, L. E. 2000. Self-organizing biochemical cycles. *Proc. Natl. Acad. Sci. USA* 97: 12503-12507.
- Orr, H. A. 2000. The rate of adaptation in asexuals. *Genetics* 155: 961-968.
- Patel, S. S., and K. M. Picha. 2000. Structure and function of hexameric helicases. *Annu. Rev. Biochem.* 69: 651-697.
- Pearce, B. K. D., R. E. Pudritz, D. A. Semenov, and T. K. Henning. 2017. Origin of the RNA world: the fate of nucleobases in warm little ponds. *Proc. Natl. Acad. Sci. USA* 114: 11327-11332.
- Phillips, R., and R. Milo. 2009. A feeling for the numbers in biology. *Proc. Natl. Acad. Sci. USA* 106: 21465-21471.
- Pinheiro, V. B., A. I. Taylor, C. Cozens, M. Abramov, M. Renders, S. Zhang, J. C. Chaput, J. Wengel, S. Y. Peak-Chew, S. H. McLaughlin, P. Herdewijn, and P. Holliger. 2012. Synthetic genetic polymers capable of heredity and evolution. *Science* 336: 341-344.
- Poon, A., and S. P. Otto. 2000. Compensating for our load of mutations: freezing the meltdown of small populations. *Evolution* 54: 1467-1479.
- Powner, M. W., B. Gerland, and J. D. Sutherland. 2009. Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions. *Nature* 459: 239-242.
- Rasmussen, B. 2000. Filamentous microfossils in a 3,235-million-year-old volcanogenic massive sulphide deposit. *Nature* 405: 676-679.
- Raynes, Y., M. R. Gazzara, and P. D. Sniegowski. 2012. Contrasting dynamics of a mutator allele in asexual populations of differing size. *Evolution* 66: 2329-2334.

- Ricardo, A., M. A. Carrigan, A. N. Olcott, and S. A. Benner. 2004. Borate minerals stabilize ribose. *Science* 303: 196.
- Robertson, M. P., and G. F. Joyce. 2012. The origins of the RNA world. *Cold Spring Harbor Perspect. Biol.* 1: 4(5).
- Rosing, M. T. 1999. ^{13}C -depleted carbon microparticles in > 3700-Ma sea-floor sedimentary rocks from west Greenland. *Science* 283: 674-676.
- Russell, M. J., and A. J. Hall. 1997. The emergence of life from iron monosulphide bubbles at a submarine hydrothermal redox and pH front. *J. Geol. Soc., London* 154: 377-402.
- Russell, M. J., A. J. Hall, and W. Martin. 2010. Serpentinization as a source of energy at the origin of life. *Geobiology* 8: 355-371.
- Russell, M. J., et al. 2014. The drive to life on wet and icy worlds. *Astrobiology* 14: 308-343.
- Schopf, J. W. 1993. Microfossils of the early Archean Apex chert: new evidence of the antiquity of life. *Science* 260: 640-646.
- Schopf, J. W., A. B. Kudryavtsev, D. G. Agresti, T. J. Wdowiak, and A. D. Czaja. 2002. Laser-Raman imagery of Earth's earliest fossils. *Nature* 416: 73-76.
- Serrano-Andrés, L., and M. Merchán. 2009. Are the five natural DNA/RNA base monomers a good choice from natural selection? A photochemical perspective. *J. Photochem. Photobiol.* 10: 21-32.
- Shapiro, R. 2006. Small molecule interactions were central to the origin of life. *Quart. Rev. Biol.* 81: 105-125.
- Silander, O. K., O. Tenaillon, and L. Chao. 2007. Understanding the evolutionary fate of finite populations: the dynamics of mutational effects. *PLoS Biol.* 5: e94.
- Silverman, S. K. 2016. Catalytic DNA: scope, applications, and biochemistry of deoxyribozymes. *Trends Biochem. Sci.* 41: 595-609.
- Sleep, N. H., K. J. Zahnle, J. F. Kasting, and H. J. Morowitz. 1989. Annihilation of ecosystems by large asteroid impacts on the early Earth. *Nature* 342: 139-142.
- Smith, E., and H. J. Morowitz. 2004. Universality in intermediary metabolism. *Proc. Natl. Acad. Sci. USA* 101: 13168-13173.
- Soga, N., K. Kimura, K. Kinoshita, Jr., M. Yoshida, and T. Suzuki. 2017. Perfect chemomechanical coupling of F_1F_0 -ATP synthase. *Proc. Natl. Acad. Sci. USA* 114: 4960-4965.
- Soll, S. J., C. Díaz Arenas, and N. Lehman. 2007. Accumulation of deleterious mutations in small abiotic populations of RNA. *Genetics* 175: 267-275.
- Stephan, W., L. Chao, and J. G. Smale. 1993. The advance of Muller's ratchet in a haploid asexual population: approximate solutions based on diffusion theory. *Genet. Res.* 61: 225-231.
- Stüeken, E. E., R. E. Anderson, J. S. Bowman, W. J. Brazelton, J. Colangelo-Lillis, A. D. Goldman, S. M. Som, and J. A. Baross. 2013. Did life originate from a global chemical reactor? *Geobiol.* 11: 101-126.
- Sturtevant, A. H. 1937. Essays on evolution. I. On the effects of selection on mutation rate. *Quart. Rev. Biol.* 12: 464-476.

- Tice, M. M., and D. R. Lowe. 2004. Photosynthetic microbial mats in the 3,416-Myr-old ocean. *Nature* 431: 549-552.
- Vaidya, N., M. L. Manapat, I. A. Chen, R. Xulvi-Brunet, E. J. Hayden, and N. Lehman. 2012. Spontaneous network formation among cooperative RNA replicators. *Nature* 491: 72-77.
- Wacey, D., M. R. Kilburn, M. Saunders, J. Cliff, and M. D. Brasier. 2011. Microfossils of sulphur-metabolizing cells in 3.4-billion-year-old rocks of Western Australia. *Nature Geoscience* 4: 698-702.
- Wächtershäuser, G. 1988. Before enzymes and templates: theory of surface metabolism. *Microbiol. Rev.* 52: 452-484.
- Wächtershäuser, G. 1997. The origin of life and its methodological challenge. *J. Theor. Biol.* 487: 483-494.
- Wächtershäuser, G. 2007. On the chemistry and evolution of the pioneer organism. *Chemistry and Diversity* 4: 584-692.
- Wagner, G. P., and W. Gabriel. 1990. Quantitative variation in finite parthenogenetic populations: What stops Muller's ratchet in the absence of recombination? *Evolution* 44: 715-731.
- Walde, P., A. Goto, P.-A. Monnard, M. Wessicken, P. L. Luisi. 1994. Oparin's reactions revisited: enzymic synthesis of poly(adenylic acid) in micelles and self-reproducing vesicles. *J. Am. Chem. Soc.* 116: 7541-7547.
- Walker, J. E. 1998. ATP synthesis by rotary catalysis. *Angew. Chem. Int. Ed. Engl.* 37: 2309-2319.
- Walker, J. E. 2013. The ATP synthase: the understood, the uncertain and the unknown. *Biochem. Soc. Trans.* 41: 1-16.
- Watt, I. N., M. G. Montgomery, M. J. Runswick, A. G. Leslie, and J. E. Walker. 2010. Bioenergetic cost of making an adenosine triphosphate molecule in animal mitochondria. *Proc. Natl. Acad. Sci. USA* 107: 16823-16827.
- Wilson, D. S., and J. W. Szostak. 1999. *In vitro* selection of functional nucleic acids. *Annu. Rev. Biochem.* 68: 611-647.
- Woese, C. R. 1967. *The Genetic Code: the Molecular Basis for Genetic Expression*. Harper & Row Publ. New York, NY.
- Wu, M., and P. G. Higgs. 2009. Origin of self-replicating biopolymers: autocatalytic feedback can jump-start the RNA world. *J. Mol. Evol.* 69: 541-554.
- Zahnle, K., L. Schaefer, and B. Fegley. 2010. Earth's earliest atmospheres. *Cold Spring Harbor Perspect. Biol.* 2: a004895.
- Zeldovich, K. B., P. Chen, and E. I. Shakhnovich. 2007. Protein stability imposes limits on organism complexity and speed of molecular evolution. *Proc. Natl. Acad. Sci. USA* 104: 16152-16157.
- Zhang, S., J. C. Blain, D. Zielinska, S. M. Gryaznov, and J. W. Szostak. 2013. Fast and accurate nonenzymatic copying of an RNA-like synthetic genetic polymer. *Proc. Natl. Acad. Sci. USA* 110: 17732-17737.
- Zhu, T. F., K. Adamala, N. Zhang, and J. W. Szostak. 2012. Photochemically driven redox chemistry induces protocell membrane pearling and division. *Proc. Natl. Acad. Sci. USA* 109: 9828-9832.

Zhu, T. F., and J. W. Szostak. 2009. Preparation of large monodisperse vesicles. *PLoS One* 4: e5009.

Figure 2.1. The series of chemical steps in amino-acid synthesis via the Strecker reaction, which requires just an aldehyde in the presence of ammonia (NH_3), hydrogen cyanide (HCN), and water. R denotes an arbitrary side chain, including that of any amino acid (the simplest being glycine with $\text{R} = \text{H}$).

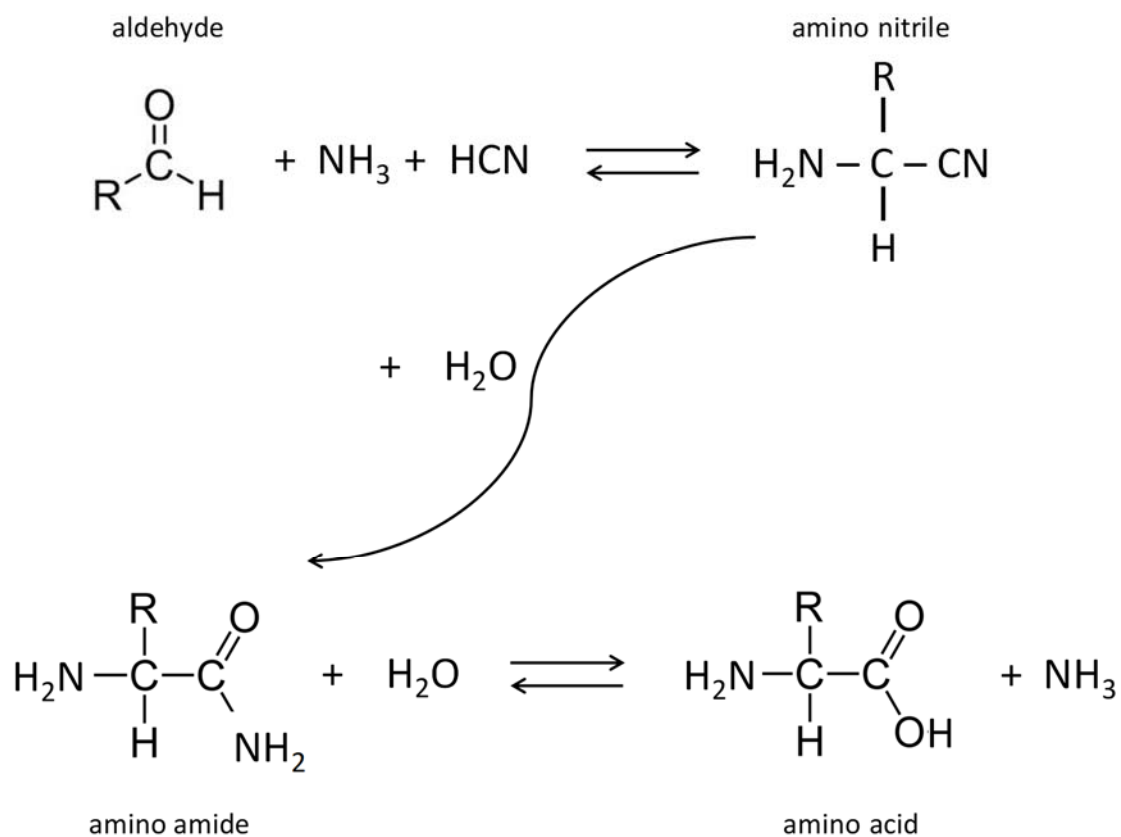


Figure 2.2. The reductive citric-acid cycle. Whereas the citric-acid cycle oxidizes sugar molecules to CO_2 and water, the reverse (reductive) cycle uses CO_2 and water to make sugars, i.e., to fix carbon. The metabolic intermediates can serve as the precursors for the synthesis of a number of amino acids (as shown in red), as in the normal citric-acid cycle (Chapter 18). This pathway, which provides a mechanism of carbon fixation in some chemolithoautotrophic bacteria, was envisioned by Wächtershäuser as a pre-biotic form of carbon fixation initiated by metal catalysts and with H_2S taking the place of H_2O as a hydrogen donor (see also DeDuke 1991). The presence of the full set of enzymes in the citric-acid cycle in all major domains of life today implies the existence of this metabolic pathway in the last universal common ancestor. However, the oxidative direction common to today's organisms (and opposite to the direction in the figure) would not have been possible in the earliest stages of life, which would have been confined to anoxic environments. Thus, the pathway may have initially run in the reductive (reverse) direction. Modified from Smith and Morowitz (2004).

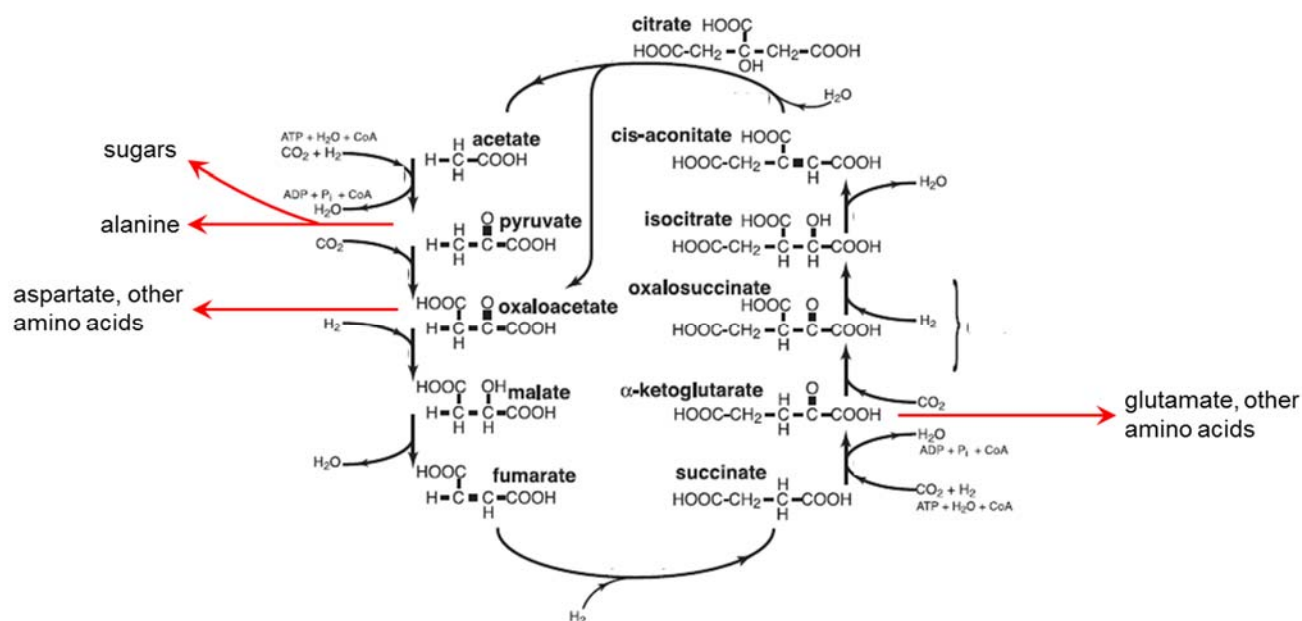
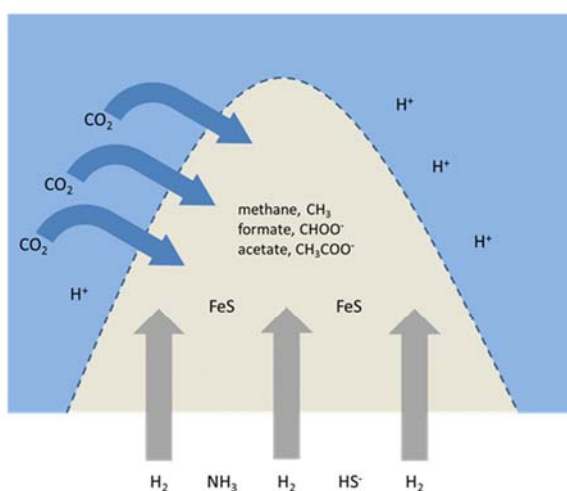


Figure 2.3. Simplified depiction of two proposed settings for the origin of life: the alkaline hydrothermal-vent hypothesis and the geothermal-field hypothesis. **Above)** Water enters the Earth's crust, where geological activity leads to the production of hydrogen, ammonia, and hydrogen sulfide ions, which are then subject to chemical reactions as they enter the mound. The latter contains pores harboring metal sulfides, which catalyze the production of simple organic compounds from CO_2 diffusing in from the overlying water. Hydrogen ions (protons) would also be subject to diffusion from the more acidic overlying water into the more alkaline environment of the mound. **Below)** Volcanic activity releases carbon dioxide, ammonia, and hydrogen sulfide gas, among other things, that accumulate in nearby ponds. The latter is postulated to contain layers of zinc and manganese sulfides, which provide both photoprotection and a mechanism for photocatalysis of simple organic compounds from CO_2 .

Alkaline Hydrothermal Vent



Terrestrial Geothermal Field

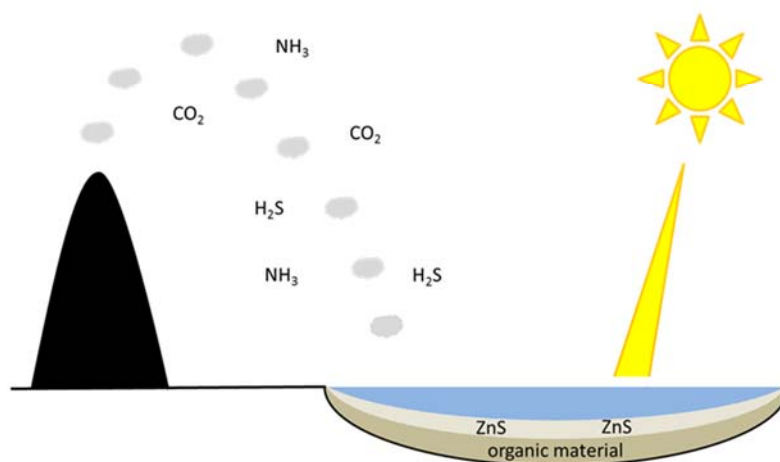


Figure 2.4. Idealized structure of ATP synthase. As a result of the export of hydrogen ions (protons) across a membrane (not shown), a proton concentration differential is maintained by cells. The resultant chemiosmotic gradient encourages a focused re-entry of protons through the ATP synthase complex, as the rest of the membrane is impermeable. The membrane-embedded rotor (containing the c subunits, and called F_0) rotates as protons pass through. This, in turn, causes the attached central rod (denoted by γ) to rotate, which activates the catalytic sites on the stationary, internal ring (with subunits α and β , and called F_1), converting ADP to ATP. This internal ring is kept stable by the stator apparatus (denoted by subunits b_2 and δ), which is anchored to the membrane with an additional structure (in light blue). After passing through the rotor, the protons exit to the inside of the membrane and are eventually re-exported, maintaining the gradient. From Boyer (2002).

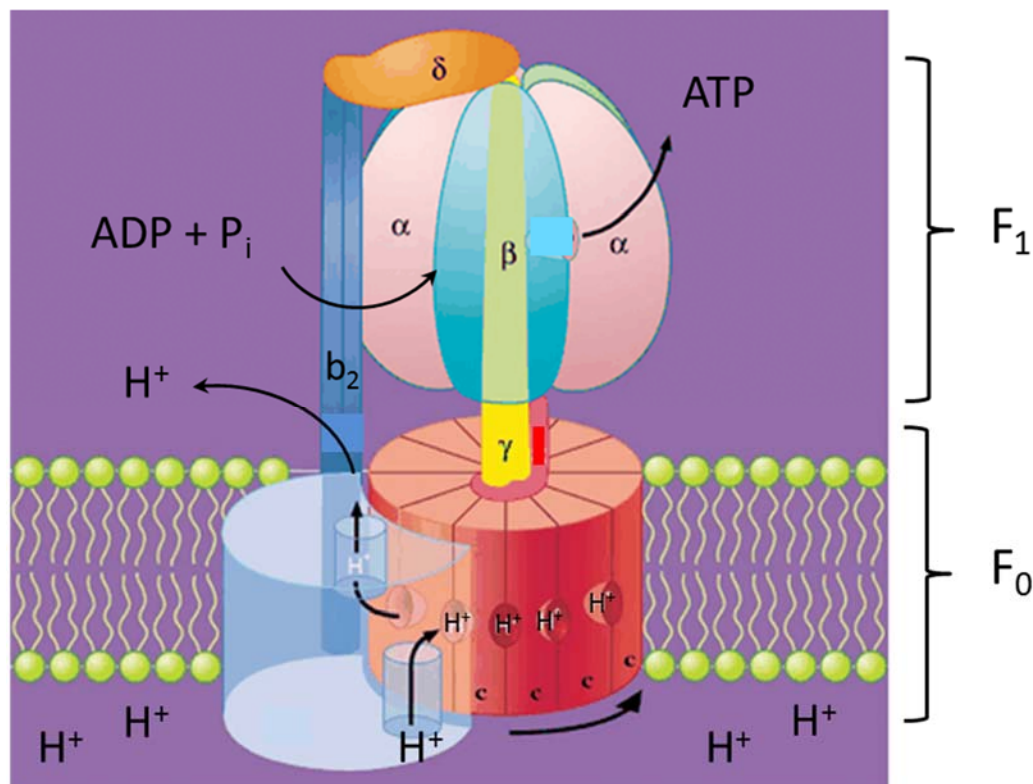


Figure 2.5. The building-blocks of DNA are derived from the conversion of RNA nucleotide precursors using the enzymes denoted to the right. Owing to the presence of a second hydroxyl group (circled in red for uracil), RNA nucleotides are more unstable than DNA nucleotides. In addition, cytosine deamination results in the production of uracil.

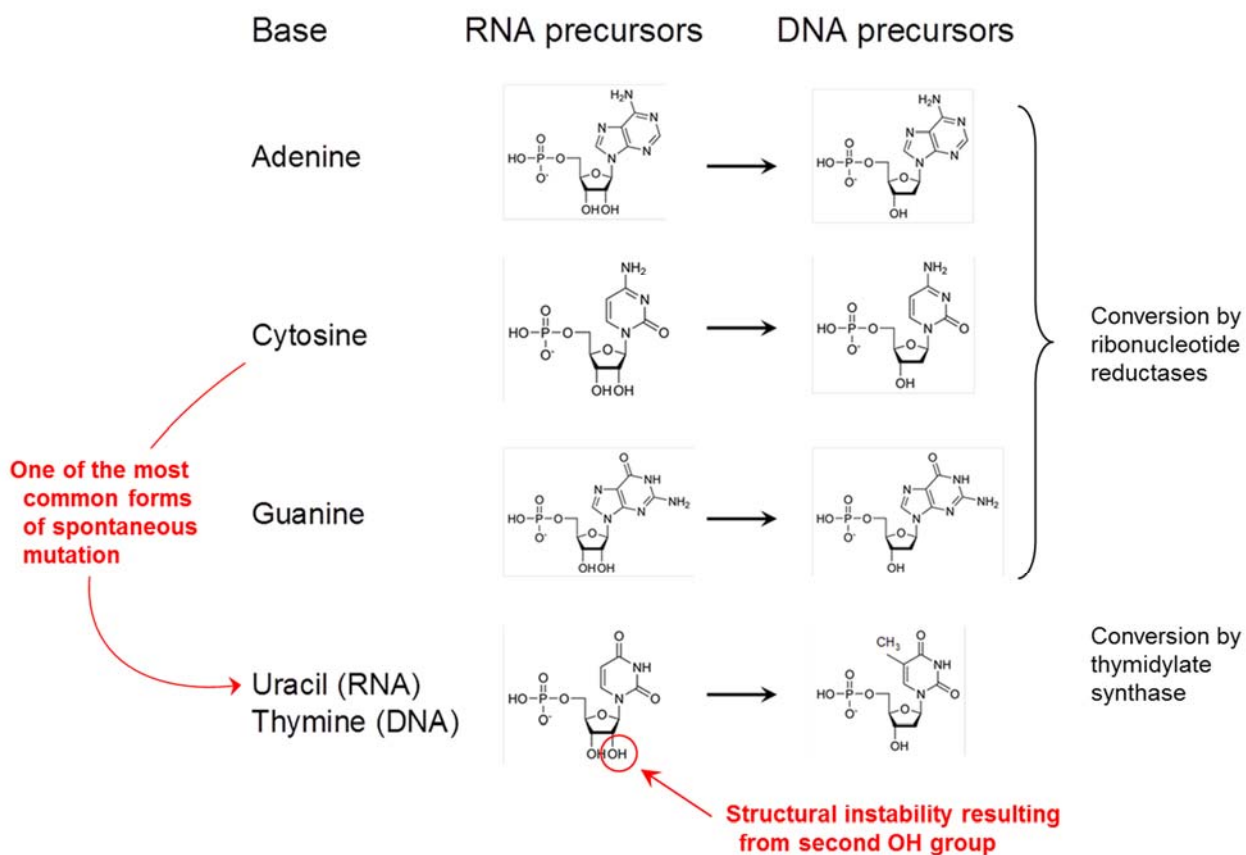


Figure 1.6. A simple tree of polymeric strings involving two alternative monomers, denoted by 0 and 1. Each parental molecule of length L can give rise to two alternative molecules of length $L + 1$ by addition of one of the activated monomers.

