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# 2. THE ORIGIN OF CELLS

15 September 2022

Ideally, a treatise that claims to be focused on cellular evolution would give substantial coverage to the earliest stages of life, so here is a bit of a letdown. Unfortunately, the cumulative effects of nearly four billion years of chemistry, physics, and geology have erased all traces of pre-cellular 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. However, this need not dampen our enthusiasm for understanding how life might have evolved. One of the goals of the active field of "origin-of-life" research is to combine our knowledge of the physical sciences and biochemistry to identify the most plausible scenarios for launching planet 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 can 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, and even then, 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. 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, and the use of ATP itself as an energy storage molecule remains an enigma. 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 frozen legacy of the singular successful lineage that gave rise to all other species on the planet?

Living systems distinguish themselves 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 its own features. Given a population of individuals with 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 so that useful variants could be maintained? 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. However, 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. This caveat should be kept in mind as the following discussion embarks on an Earth-first view, but we are still confronted with the same fundamental 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[14,68,125,154]. Because some of the more massive impacts generated enough heat to sterilize the entire planet, it is generally thought that the roots of life must be younger than 3.8 BYA. This does not rule out the possibility that biology experienced a number of false starts prior to this point, and small graphite inclusions (with carbon isotope ratios compatible with a biological origin) have been found in rocks dating to 4.1 BYA[8].

Further refinement of this key point in time 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[49,144,162], and the oldest known fossils, some from filamentous organisms and potentially eukaryotic in nature, date to 3.5 to 3.2 BYA[16,77,140,148,149,164].

Our understanding of biology's reliance on a limited set of 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 plausible scenarios requires knowledge of bioenergetic, geochemical, and physical opportunities and constraints in the prebiotic world. For example, given that car-

bon in the early Earth's atmosphere was dominated by oxidized forms ($CO_2$ and CO resulting from volcanic out-gassing), some source of sustained external energy would have been required for the construction of reduced-carbon compounds (containing C-H bonds) 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[27]. 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[107,108]. Based on the assumption that the earliest atmosphere harbored methane, ammonia, hydrogen, and water, he sealed these four compounds into a sterilized glass apparatus, which was then subjected to cycles of electrical discharges cooling periods to condense the resultant products. A few days of such treatment yielded 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[67].

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 yields the spontaneous production of the simplest amino acid, glycine (Figure 2.1). Similar reactions starting with more complex aldehydes (having side residues to the -CHO group other than the H in formaldehyde; Figure 2.1) lead to other amino acids[9]. Carbohydrates are much less stable than amino acids, but plausible scenarios for their accumulation have also been suggested. For example, when in complex with borate, carbohydrates are stabilized and differentially channeled towards pentoses such as ribose[75,142], a potentially important insight given that ribose is a key component of RNA.

Although the Miller-Urey experiments reinforced the popular idea that life emerged spontaneously out of a so-called primordial soup[61,130], a number of doubts have been raised about the prebiotic-soup hypothesis[95]. 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) being the primary nitrogen source[177]. A second issue is that open water is counterproductive to the maintenance of the organic aggregates essential to the nucleation of life. 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[8]. However, it is unclear that such sporadic delivery could provide the sustenance for emerging life forms relying on inefficient biochemical pathways (not yet refined by natural selection).

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 an aid, not a hindrance. Although there are still many unresolved issues, 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.

These are not simple issues, but Wächtershäuser[165–167] suggested a plausible autotrophy-first scenario for the emergence of metabolism. He envisioned a particular kind of chemoautotrophy naturally generated in a high-pressure, high-temperature environment associated with underwater volcanic activity, an idea first broached by Baross and Hoffman[5]. Assuming the presence of one-carbon compounds such as CO, $CO_2$, COS, HCN and $CH_3SH$, and with iron and nickel sulfides acting as catalysts, a sort of primitive form of spontaneous carbon fixation was postulated. A specific reaction that Wächtershäuser had in mind was the oxidation of FeS with $H_2S$ to produce $FeS_2$ (pyrite), hydrogen ions (protons), and electrons. The products of this reaction were proposed to drive the reduction of $CO_2$ to formate ($HCO_2$), and then to more complex chemicals such as acetate ($CH_3CO_2$) and pyruvate ($CH_3COCO_2$).

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 $CO_2$ into a reverse form of the citric-acid cycle (whose forward reaction is used in today's organisms to break down sugar; Figure 2.2). Under his model, primordial energy transduction would have been accomplished by thioesters rather than by the phosphate-bearing molecules essential to today's organisms, i.e., the hypothesized intermediate metabolites were thiol analogs (containing -SH groups) of the components of today's citric-acid cycle, with $H_2S$ (rather than the usual $H_2O$) entering at various steps. Based on biochemical considerations, the very high $CO_2$ concentration in hydrothermal-vent environments provides a kinetically favorable setting for running the citric-acid cycle in reverse[158]. Thus, it may be no coincidence that a core of today's metabolism operates by using enzymes enriched in iron-sulfur complexes[56].

The existence of modern hydrothermal-vent microbes dependent solely on a continuous flow of chemistry and energy demonstrates the ability of such environments to support life. Although this need not imply that the current inhabitants of such environments have been derived in a linear line of descent from the time of life's origin, some aspects of Wächtershäuser's model have been validated[28]. Laboratory experiments imposing hydrothermal vent-like conditions do yield pyruvate in the presence of transition-metal sulfides[28], and reactions involving pyruvate can lead to more complex organic molecules, some of which have properties related to the lipids essential to building membranes[63]. At temperatures compatible with life, pyruvate reacts with $H_2S$, $H_2$, and $NH_4$ to yield products such as aldols, lactate, alanine, proprionic acid, and sulfur-containing organics, with the specific blends of products being strongly dependent upon temperature and the mineral substrates[126]. Starting with the metabolites of glycolysis and the pentose-phosphate pathway (Chapter 19), almost the full set of reactions of these pathways can be generated in a completely abiotic setting[74], and a mixture of pyruvate and ferrous iron yields nine of the eleven

intermediate metabolites of the citric-acid cycle[110].

The key point here is that simple 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. 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. Under this view, modern-day enzymes would have then emerged secondarily as catalytic enhancers of pre-existing abiotic 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 by Cody[28] and Stüeken et al.[160] 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 those 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 secondarily, only after the establishment of ion-tight membranes.

Two of the more 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 a potential source of geothermal energy and chemistry envisioned by Wächtershäuser[31]. However, their short longevity, extremely low pH, and lack of compartmentalization prompts questions over their suitability as cradle-of-life candidates[79,100,145].

Less extreme variants include lower-temperature, alkaline hydrothermal-vent systems[99,146,147]. 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. Dissolved $CO_2$ plays the latter role in such environments, entering from above and then being reduced to simple hydrocarbons such as methane, formate, and acetate. $N_2$ is also reduced to $NH_3$, and sulfates to $H_2S$, providing potential paths for the downstream integration of nitrogen and sulfur into organic compounds. Alkaline-hydrothermal vents also 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. Based on these observations, like Wächtershäuser, 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[80].

There are other attractive aspects of alkaline hydrothermal vents as potential locales for the origin of life. First, they present a 10,000-fold proton gradient, as the pH for the ocean-water influent $\simeq 6.0$ while that for the effluent $\simeq 10.0$. 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 iron and nickel sulfide groups envisioned as operating in early abiotic metabolism also comprise the catalytic sites for energy transfer in the electron-transport chain deployed in today's organisms[22,62]. Third, abiogenic production of amino acids has been detected within such systems[106].

Notably, one of the six known biological mechanisms of carbon fixation, the acetyl-CoA pathway (Chapter 19), has similarities to the pathway invoked in the alkaline hydrothermal-vent hypothesis[100]. The acetyl-CoA pathway is the only known carbon-fixation mechanism that yields energy 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, $N_2$, and $H_2S$. These two lineages utilize apparently unrelated enzymes in the acetyl-CoA pathway and produce different final products (methane vs. acetate), suggesting an ancient episode of parallel evolution[80]. 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 in the following chapters, there are a number of other ways in which prokaryotes 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 processes (hot springs and geysers) associated with volcanic activity harbor most of the advantages envisioned by the hydrothermal-vent hypothesis as well as others[34,113,114,117]. Under this alternative view, life would have originated in shallow ponds, with solar irradiation being the primary energy source. The condensates found in such ponds likely would have been enriched with potassium, metal sulfides, zinc, and boron, with phosphorus concentrations possibly as much as 100× greater than those at hydrothermal vents. Assuming regularly occurring wet/dry cycles, concentrations of many other reagents would have been elevated as well.

The geothermal-field hypothesis is inspired, in part, by one of the great mysteries in cell biology – the relative use of various cations relative to their environmental abundances. Intracellular concentrations of potassium, zinc, manganese, and phosphate (and most other elements; Chapter 7) are generally higher than those found in modern sea water, whereas the opposite is true for sodium[115]. To achieve intracellular disparities in ion concentrations, modern cells invest considerable energy in the operation of ion pumps (Chapter 18), raising the question as to whether the differential cellular use of potassium and sodium is a historical relic. Because sodium concentrations are low in geothermal fields relative to the situation in marine environments, the geothermal-field hypothesis provides a potential explanation for this conundrum. 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

likely would not have been the case on the surface of an early Earth devoid of oxygen, as the $H_2S$ associated with geothermal activity would not have been oxidized to sulfuric acid. Second, metal sulfides likely would have precipitated in shallow waters. Notably, ZnS crystals are powerful photocatalysts, raising the possibility that diverse hydrocarbons might have been produced by a sort of abiotic photosynthesis in shallow, light-rich environments. Third, as they are highly efficient at scavenging UV light, ZnS and MnS crystals also have photo-protective capacity. Thus, the primary architects of this hypothesis, 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 various enzymes (which attempt to pinpoint their first appearance in the Tree of Life) suggest a very early origin of those utilizing Zn, Mn, $H_2S$, and K (but not Na)[35,43].

Deamer and Georgiou[37] 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. It should be emphasized, however, that 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[64]. This is an obvious matter of concern with respect to the origin of an information-bearing genome, and the well-known instability of complex molecules at high temperatures. The geothermal-field hypothesis is also confronted with an additional issue – the possibility that the entire Earth was under water at the time of life's origin[42].

## 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 starting points do not meet our definition of life, although they do offer potential explanations for a number of cellular features. Nonetheless, regardless of which, if any, of these suggested links to the past is correct, abiotically induced chemical reactions must have been taken over eventually by protein catalysts. And therein lies the problem. The spontaneous assembly of complex proteins from environmental sources of amino acids is extraordinarily unlikely, and the refinement of catalytic properties in the absence of a replicating genome, required for the operation of natural selection, is even less likely. Because all modern cells 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 would be a set of molecules that served 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 is generally catalytically inert. RNA is the only biomolecule in today's cells for which some variants can both specify a genotype and express a phenotype. This then leads to the suggestion that RNA

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, genetic continuity was assured by the replication of an RNA-based genome, with all underlying catalysis also being carried out by RNAs, and no involvement of proteins or DNA[32,54,64,131,143,174]. Under this view, the complex protein repertoire now employed by all organisms arose secondarily, layered on top of the more fundamental RNA scaffold.

A number of observations provide indirect support for this hypothesis. First, 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 and facilitating amino-acid chain formation. Third, across the Tree of Life, all of the major players in today's 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 under selection for a wide variety of catalytic activities[72,173].

If there was an early RNA World, it may have coexisted with and even exploited one of the physical energy-generating scenarios outlined above. Successful members of the population of RNA molecules might then have gradually evolved a coding mechanism for producing proteins for enhancing the efficiency of energy harvesting, and eventually displacing the abiotic pathway entirely. Provided that 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 (i.e., natural selection) on the path to what we now call life. Under this view, just as we see the reliance on a proton-motive force and on particular elements as being evolutionary relics, the diverse roles played by RNA in today's cells, especially in transcript processing and translation, can be viewed as molecular descendants of this early era of biochemistry.

There remains the fundamental question of how an information-bearing system can get started before the arrival of a platform for exploiting information. One simple scenario is outlined in Foundations 2.2, where it is shown that provided there is a mechanism of polymerization and recurrent input of alternative monomeric building blocks, an equilibrium population of polymers with variable lengths and sequences 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. What remains, however, is the need for a genetic and cellular mechanism for the production and maintenance of heritable variation essential to the operation of natural selection.

Although these observations support the plausibility of an RNA-world episode in the origin of life, it should be kept in mind that the RNA World is a hypothesis, not a confirmed fact. One central caveat is that it is now known that like RNA, DNA can take on a number of catalytic functions (when maintained in single-stranded form, but allowed to fold into stems and loops); these include RNA cleavage, RNA and DNA ligation, and conjugation of amino acids to nucleotides[153].

Other unresolved issues leave room for doubt about the extreme model in which RNA is the only replicator and the only catalyst[103,151]. 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[7,143], and ribose might also have arisen in the environments envisioned in the hydrothermal-vent and geothermal-field hypotheses, especially if boron was present[75,103,124,135]. 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[7,124,139]. The speed of such reactions is not impressive, but early life had the luxury of time and perhaps 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, not *de novo* from inorganic materials.

Second, jump-starting an RNA World would not only require a pool of ribonucleosides, but a means for activating them with pyrophosphates to promote chain growth. A mechanism to polymerize these basic building blocks would also be required. Moreover, to maintain continuity across generations, self-replicators would need to avoid accidental replication of other competing genomes, which would stifle the process of natural selection.

Third, although key steps towards self-replication have been accomplished[86,109], a fully self-replicating ribozyme has not yet been developed, despite considerable effort. One could argue that this is a minor concern, as the emergence of life had eons of time in which to discover a few exceptional molecules. This dwarfs the three decades of research performed in a handful of laboratories in 10-ml test tubes, $\sim 10^{23}$ of which would be required to match the mass of oceanic water. Indeed, recent laboratory experiments involving thermal gradients[101] or ice substrates[3] have succeeded 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[10].

Confronted with these numerous requirements for the assembly and maintenance of ribonucleotide polymers, some have suggested a pre-RNA World dominated by some other polymer capable of genetic and catalytic functions[73,132,151]. There is no shortage of candidate molecules. For example, numerous nucleotide analogs substitute various moieties (including amino acids) for ribose[143], and some of these are capable of nonenzymatic template copying[137,179]. The abiogenic production of hybrid molecules consisting of RNA pyrimidines and DNA purines has even been demonstrated[176]. 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[41,116,150].

Two strong arguments favor DNA arising after both RNA and proteins. First, an early RNA-Protein World requires the existence of a genetic code prior to the arrival of DNA, which is consistent with the ubiquitous use of transfer, messenger, and ribosomal RNAs in translation. Second, modern cells derive their DNA building blocks (deoxyribonucleotides) via chemical modifications of ribonucleotides. Ribonucleotide reductases are used in the production of dAMPs, dCMPs, and dGMPs, while thymidylate synthase produces dTMPs by methylating dUMPs (Figure 2.5). Remarkably, the two primary prokaryotic lineages, the bacteria and the archaea (Chapter 3), utilize seemingly unrelated thymidylate synthases[123] as well as two apparently unrelated sets of DNA-replication proteins[44,85,129]. 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 a pre-DNA World, why would the transition to a DNA World be so complete as to eradicate all RNA-based genomes (other than RNA viruses) from the cellular domains of life? One attractive answer invokes two chemical features that enhance the stability 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 spontaneous cytosine deamination, which produces uracil. In thymine-bearing DNA, uracil can be recognized as aberrant and corrected prior to replication (once a mechanism for such recognition is established), but such a distinction is impossible in RNA. Thus, an organism that discovered a way to store its genome as DNA while retaining RNA for nonheritable phenotypic functions would have had a substantial advantage in terms of reliable genome propagation.

### 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. For without discrete individuals, the efficiency of natural selection would have been greatly diminished as any key metabolites produced by a local entity would become public goods, eliminating the genotype-phenotype loop. On the negative side, cellularization reduces access to resources, although the earliest membranes might have been quite permeable.

The most compelling reason to think that the most recent common ancestor to all of today's life had a cell envelope is the universal use of membrane-bound ATP synthase (noted above) as well as several other membrane-associated proteins[65,119]. 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, from which lipids are built, have been found in extraterrestrial rocks such as the Murchison meteorite[36], and can

be synthesized under plausible prebiotic conditions. For example, starting with CO, $CO_2$, and $H_2$ gases in the presence of metal catalysts at high temperature, a reaction known as Fischer-Tropsch synthesis can lead to long-chain fatty acids[104]. Second, lipids spontaneously assemble into organized bilayered vesicles, with their hydrophobic tails and hydrophilic heads pointing to the inside and outside of the vesicle, respectively (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.

Of course, 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, using a simple system containing protocells made out of lipid bilayers, some empty and some containing RNA, Chen et al.[24] showed 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. Further work has shown that in a setting containing DNA templates and DNA primers, polymers can grow within a membrane-bound vesicle permeable to charged nucleotides, again causing osmotic pressure and vesicle growth[98,169].

Once such a system like this was in place, any cell that contained a faster replicator would experience still more rapid growth, thereby initiating a process of natural selection. There remains, however, the question of how membranes might come to be associated with nucleotides at all. Black et al.[12] 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 mechanisms by which: repeated rounds of nucleotide polymerization might have been achieved; competition between strand reannealing and new chain growth might have been avoided; and an organized mode of cell division might have been established. 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[181] 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[180].

From observations on these simple kinds of systems, 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. However, the short-chain fatty-acid membranes employed in these studies are highly permeable to a wide variety of nutrients, including amino acids and nucleotides. In contrast, 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[17] found that two-chain 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 still another issue – by conferring reduced permeability, the progressive establishment of a phospholipid membrane would diminish access to the external environment. In principle, the gradual emergence of the phospholipid membrane may have fostered the evolution of internal cellular biosynthetic pathways to compensate for the reduction of external resource availability. However, such an argument is not particularly compelling from an evolutionary perspective, as any realistic scenario for this sort of transition would have required a series of steps in which cellular fitness was never diminished. Natural selection allows populations to increase fitness in response to selective challenges, but does not encourage the continuous exposure to harmful conditions.

Finally, it should be noted that all of the preceding views on the origin of membranes are based on the assumption that the cytoplasm of protocells resided inside cell membranes. An alternative view considers the opposite topology, with the cytoplasm initially nucleating on the outside of vesicles called obcells, perhaps being held in place by actin-like filaments[13,23,59]. 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. Although perhaps not impossible, these kinds of scenarios for the start of life ignore the central requirement for individuality to enable the efficient operation of natural selection.

## 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. Both camps put a priority on framing hypotheses based on presumed geochemical / biophysical constraints, an entirely reasonable and desirable enterprise. However, remarkably absent from this debate is the equally important matter of the maintenance of a population with heritable features capable

of progressive adaptation. Indeed, most origin-of-life research has proceeded with almost no consideration of fundamental evolutionary processes.

To ensure a sustainable and productive path forward by natural selection, an information-bearing molecule must be capable of generating accurate copies of itself. However, at the dawn of life, replication fidelity would have been much less accurate than in today's refined organisms, imposing a significant restriction on genome size. Given its relative chemical stability, the arrival of DNA might have provided a more permissive environment for genomic expansion and hence the emergence of more complex biological functions, but there still must have been substantial limitations.

In the first attempt to grapple with this issue, Eigen[45,46] 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[19]. Theory from population genetics (Foundations 2.3) shows that for a given genome size, extinction avoidance requires a sufficiently high replication fidelity that enough mutation-free offspring genomes are produced each generation to avoid progressive fitness 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 proportion of mutation-free individuals in a very large population in selection-mutation balance is $e^{-\phi}$ (Foundations 2.3), so if $\phi$ 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. Indeed, this general principle underlies the application of lethal-mutagenesis strategies for eradicating pathogens[19–21,25,66]. Using laboratory populations of RNA molecules with an error rate $> 10^{-5}$ per nucleotide site, Soll et al.[157] showed that the time to extinction is positively related with population size, as theory predicts.

The concept of mutational meltdown is based on the inevitable consequences of sampling of propagules from one generation to the next[92,93]. 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 probability of loss is still substantial, 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 high 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 population-size reduction progressively increases the probability of loss of the best-class individuals, with each such loss leading to still further loss in fitness.

Mutational-meltdown theory (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 (the magnitude of which is related to the reciprocal of population size; Chapter 4), no further improvement is possible. As a first-order approximation, for example, for a small population with $10^4$ individuals, $U_d$ cannot evolve to a level much lower than $10^{-4}$. Thus, because $U_d$ is approximately equal to the product of the mutation rate per site and the number of nucleotide sites in the genome with functional effects, 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[58,138,152,168], but sufficiently high mutation pressure will still eventually lead to a mutational meltdown[25,178].

The relevance of these results to understanding the origin of life is that levels of replication fidelity were likely quite low in the earliest stages of life. For example, polymerization off an RNA 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[3,71,179], many orders of magnitude higher than in any of today's cells (Chapter 4). 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[2,64,163]. However, the salient point remains – a resolution of population-genetic issues is just as critical to understanding the origin of life as a focus on promising geological settings.

**Summary**

- The basic properties of life were established on Earth $\sim 3.5$ 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.

- Plausible hypotheses identify certain environmental settings on an early Earth that might have been conducive to the spontaneous emergence of the ancestor of all of today's organisms. However, the commonly invoked open-water primordial soup is not one of them. The peculiar sets of reactions, metal cofactors, and metabolic building blocks that life came to depend may be a reflection of the ancestral setting.

- Given the core roles played by RNA in several key functions in today's organisms, the RNA-world hypothesis promotes the view that a single type of molecule (RNA) simultaneously provided the 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. Demonstrations of the plausibility of single steps towards life in restricted chemical/physical environments too often lead to increased adherence to specific views on the nature and order of events leading to the origin of life. This weaving of entire series of low-probability events into a convincing, comprehensive scenario should be interpreted with caution.

- Major downstream challenges in the origin of life would have involved the establishment of reliable means of genome transmission, which requires membranes for individuality, accurate RNA/DNA polymerases for replication fidelity, and a platform for the production of catalytic machinery consisting of proteins.

- Putting aside the shortcomings in our understanding of the specific steps towards the establishment of life, considerable 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 cell growth, 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[94,136] (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 on chemiosmosis to regenerate 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 (generally denoted as 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[79]. 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. An additional implication is that the use of ATP as an energy carrier emerged prior to the evolution of ATP synthase.

ATP synthase is a complex molecular machine, generally consisting 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 (about 10× more rapidly than the rotation of the wheels of a car moving at 60 miles/hour). Synthesis of ATP from ADP occurs as the rotating stalk interacts with the stationary $F_1$ ring carrying the catalytic subunits[171].

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[33,118,120]. Owing to the complex structure of ATP synthase, the mechanisms of its origin are far from clear. The probability of the sudden *de novo* evolutionary emergence of such a 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[170]; a relationship to the membrane-bound motor of the bacterial flagellum has also been suggested[120] (Chapter 16). Similarities also exist between the internal

catalytic subunit ($F_1$) and the ring-like helicases that use energy from ATP hydrolysis to separate the strands of DNA[134,170].

For these changes to have become established without the loss of ancestral gene functions remains unclear, some involvement of gene duplication and reassignment seems almost certain (Chapter 5). Indeed, the catalytic component ($F_1$) 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[156]. 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[172]. For these species, the cost of each ATP is just $8/3 = 2.67$ protons. Notably, the stoichiometry of the ($F_0$) ring is conferred by the sequence of the monomeric subunit (c), so that gene transfer from one species to another maintains the donor structure. This enables the experimental analysis of shifts in gear structure[102,175].

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[171]. 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[171], 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.

These observations raise the question as to why all species don't utilize a system with the efficiency of that in the mammalian mitochondrion. But 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[4]. Members of the Chlamydomonadales (a group of green algae) have nine unique stator proteins[81], and *Euglena* ATP synthase has eight unique subunits[112]. Some of these modifications have effects on the higher-order assembly of ATP synthases into multimeric clusters in mitochondria[48,111]. Many other differences are known in the bacteria and eukaryotes[122,171].

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 ATP synthases couple the synthesis / hydrolysis of ATP with the transport of sodium ions rather than protons, and some are capable of using both. The interwoven phylogenetic relationships of the Na- and H-utilizing enzymes[120] leave the ancestral state ambiguous.

Thus, 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. Why the use of membrane-bound

machine, and 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)? Does the use of Na as a driving ion imply anything about the context in which ATP synthase originated?

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**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[127] 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 types of 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 elongation reactions:

$$i + *0 \rightarrow i0$$
$$i + *1 \rightarrow i1$$

occurring at rates $a_{i0}$ and $a_{i1}$ (the absence of a $*$ indicates a recipient molecule). 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

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

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,

$$n_i = b_i b_{i'} b_{i''} \cdots, \tag{2.2.2}$$

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 unique 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 $\lambda$); 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

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

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 $\lambda/d$, 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[175]. More details on all of these matters and others can be found various overviews[11,96,97,127,128].

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**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 4). Here, we apply some basic results from population-genetic theory to gain insight into the fundamental features of genomic stability essential for the establishment of life. 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 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

$$W_k = (1 - s)^k, \tag{2.3.1}$$

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 $k$. 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[60].

Muller[19] (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 ultimately 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[47], and numerous authors have attempted to solve the difficult problem of the rate at which the ratchet clicks[50,53,57,159]. 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[92,93].

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 $U_d/s$ being the average number of deleterious mutations per individual[60]. The Poisson distribution is a simple function of the mean, with the expected number of individuals in the lowest ($k = 0$) class being

$$N_0 = Ne^{-U_d/s}, \tag{2.3.2}$$

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

$$U_d \ll s \ln(sN). \tag{2.3.3a}$$

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

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

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[78].

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 $\Delta U_d$ 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[69,76,87].

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

$$U_d^* = 1/N. \tag{2.3.4}$$

This follows because the power of drift can be no smaller than $1/N$, and from the above-point that selection is ineffective if its magnitude is lower than the diffusive power of drift[88,89].

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 follow 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

$$N^* = 1.76/s. \tag{2.3.5}$$

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 $s$ may be on the order of 0.001 to 0.01 for fitness-altering mutations[91]. 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 individual 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[90]. 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. Third, virtually all populations behave genetically as though they are much smaller than their absolute sizes, owing to the selective interference that operates when multiple mutations are simultaneously competing for promotion by natural selection (Chapter 4).

The previous results rely on the assumption that essentially 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[18,52,83,84,133], but because selection operates on the immediate time scale (rather than looking to the future), it is unclear whether such rates are ever achievable[26,40,70,161]. 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[39,55,82]. However, such events are generally transient, as they are quickly followed by reversion of the mutation rate[1,51,141]. 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[89,90].

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**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 (the simplest for an amino acid being R = H for glycine).

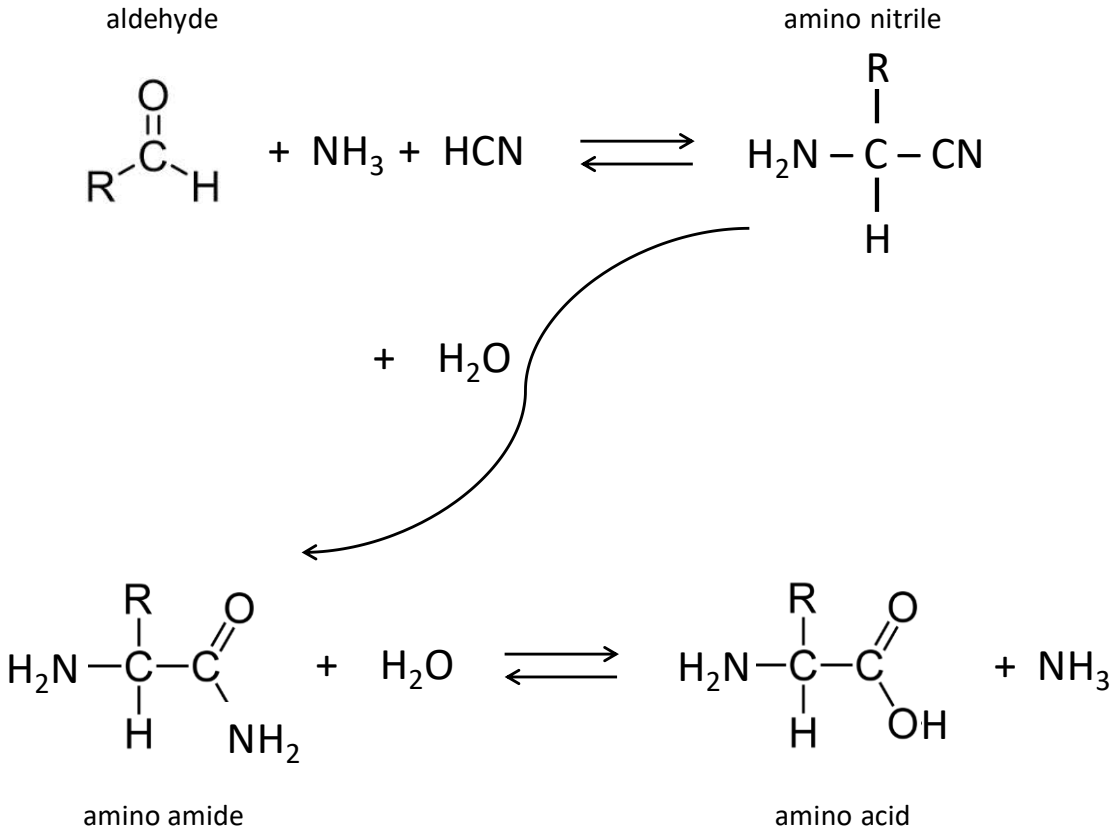

**Figure 2.2.** The reductive citric-acid cycle. Whereas the citric-acid cycle oxidizes sugar molecules to $CO_2$ and water, the reverse (reductive) cycle uses $CO_2$ and water to make sugars, i.e., to fix carbon. In principle, the metabolic intermediates can serve as the precursors for the synthesis of a number of amino acids (as shown in red), as in the normal citric-acid cycle (Chapter 19). This pathway, which occurs in some chemoautotrophic bacteria, was envisioned by Wächtershäuser[165–167] 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 DeDuve[38]). The presence of the full set of enzymes in the citric-acid cycle in all major domains of life today implies the existence of the citric-acid cycle 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 emerged in anoxic environments. Thus, today's citric-acid cycle may have initially run in the reductive (reverse) direction. Modified from Smith and Morowitz[155].

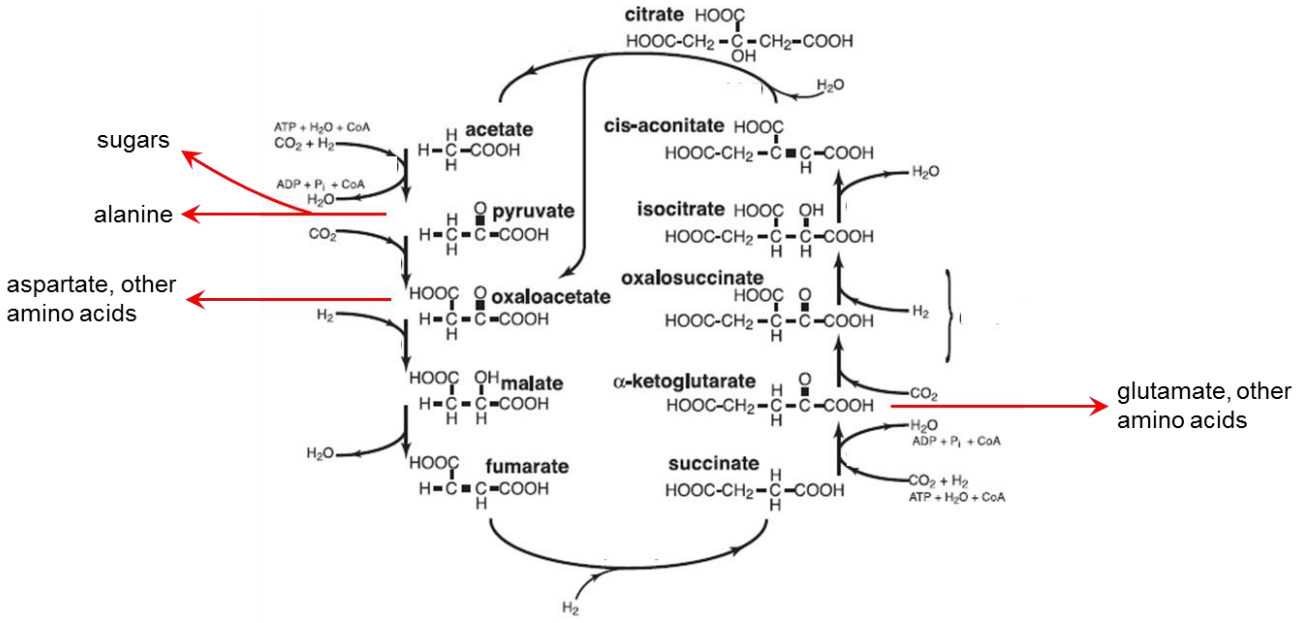

**Figure 2.3.** Simplified depiction of two proposed settings for the origin of life. **Above**) The alkaline hydrothermal-vent hypothesis. Water enters the Earth's crust, where geological activity leads to the production of hydrogen, ammonia, and hydrogen sulfide ions, which are then subject to chemical reactions as they enter the overlying 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**) The geothermal-field hypothesis. 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 $CO_2$.

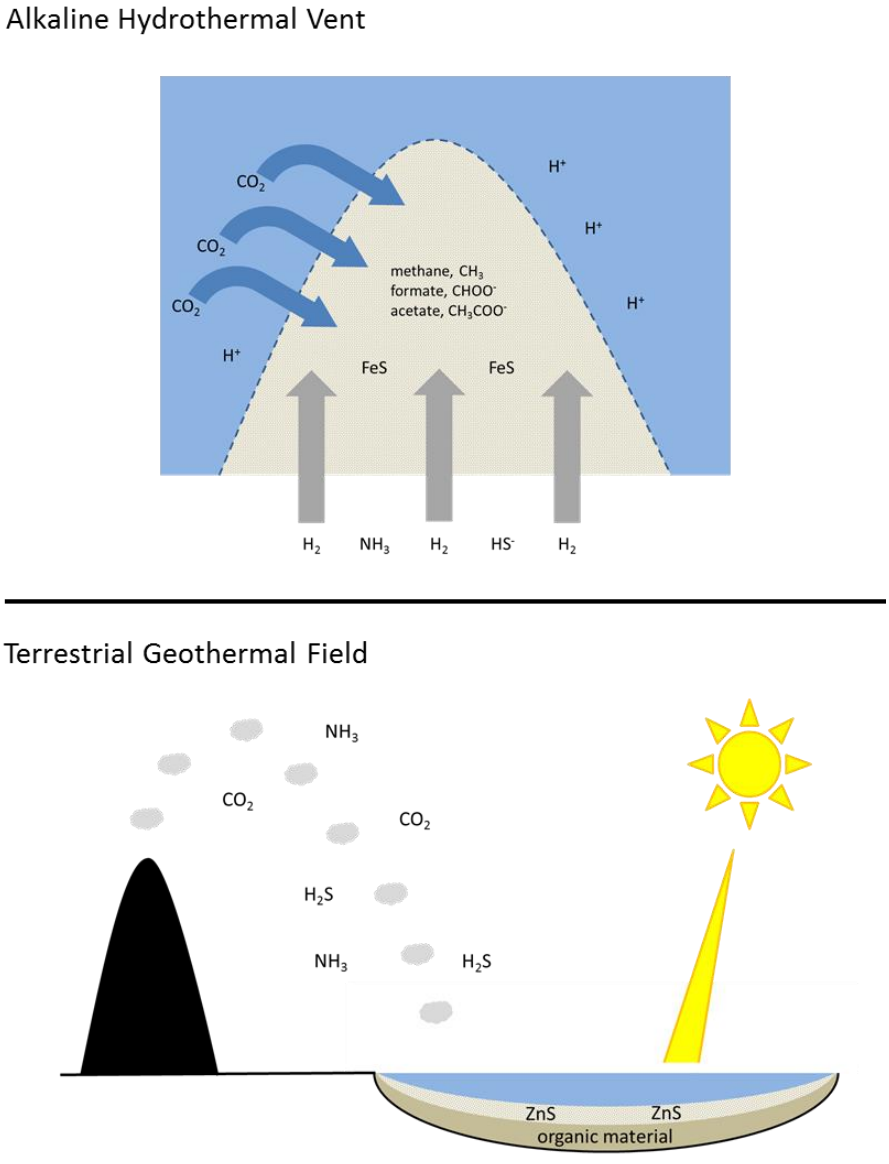

**Figure 2.4.** Idealized structure of ATP synthase. As a result of the export of hydrogen ions (protons) across a membrane (not shown), a proton concentration differential is maintained by cells. The resultant chemiosmotic gradient encourages a focused re-entry of protons through the ATP synthase complex, 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 $\gamma$) to rotate, which activates the catalytic sites on the stationary, internal ring (with subunits $\alpha$ and $\beta$, and called $F_1$), converting ADP to ATP. This internal ring is kept stable by the stator apparatus (denoted by subunits $b_2$ and $\delta$), which is anchored to the membrane with an additional structure (shown in light blue). After passing through the rotor, the protons exit to the internal side of the membrane and are eventually re-exported, maintaining the gradient. For prokaryotes, the interior refers to the cytoplasm; for eukaryotes, it refers to the inside of the mitochondrion. From Boyer[15].

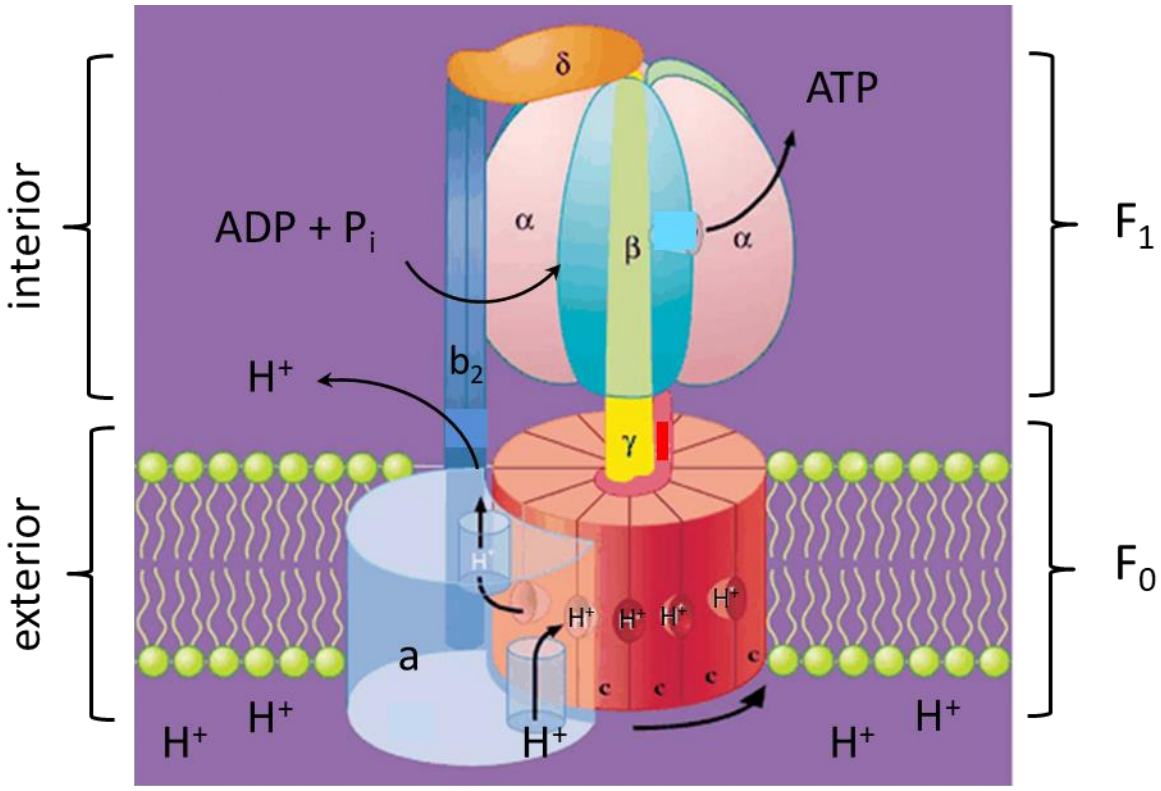

**Figure 2.5.** The building-blocks of DNA are derived from the conversion of RNA nucleotide precursors using the enzymes denoted to the right. Owing to the presence of a second hydroxyl group (circled in red for uracil), RNA nucleotides are more unstable than DNA nucleotides. In addition, cytosine deamination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

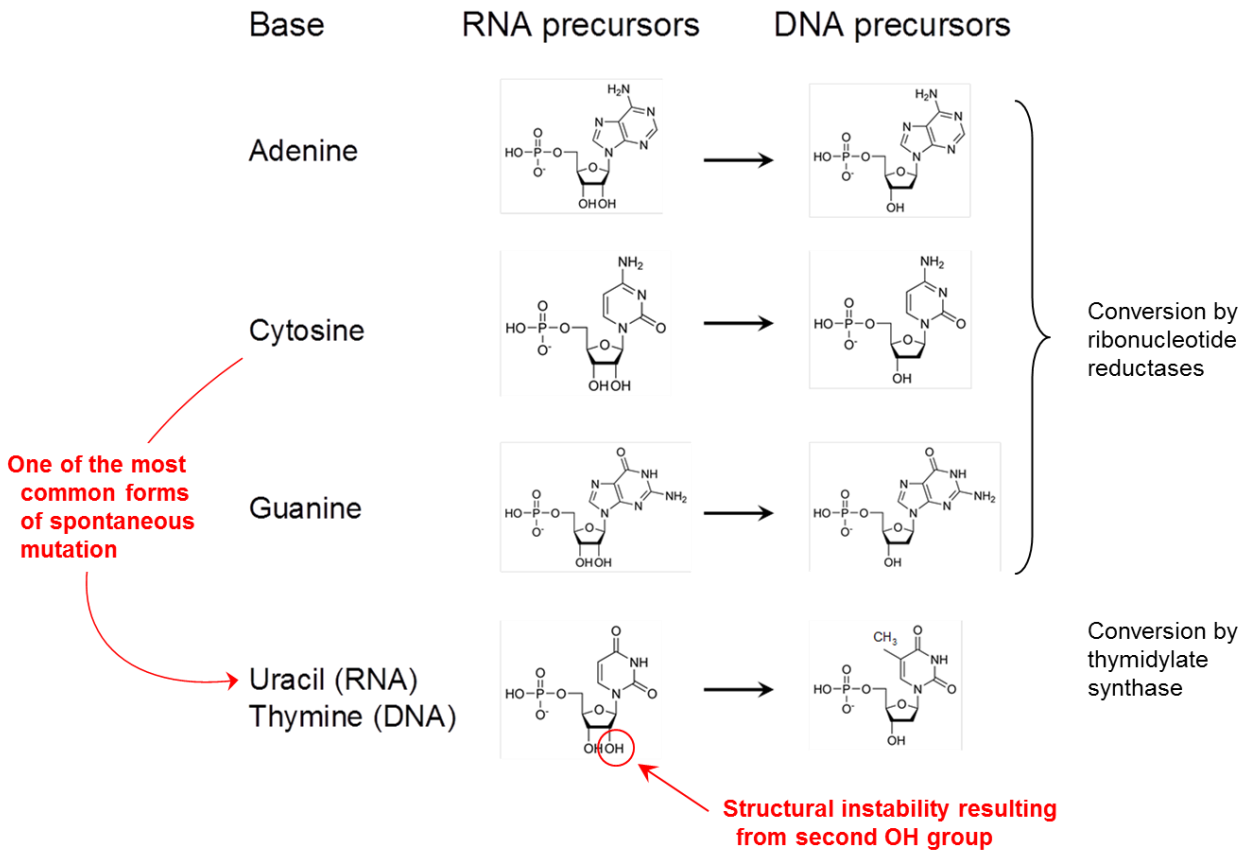

**Figure 2.6.** A simple tree of polymeric strings involving two alternative initial monomers, denoted by 0 and 1. Each parental molecule of length $L$ can give rise to two alternative molecules of length $L + 1$ by addition of one of the activated monomers.