ABSTRACT

Beyond the Cradle: Mechanisms and Applications Towards the Colonization of Intragalactic Exoplanets

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Since the dawn of humankind, people have looked to the sky, wondering what lies beyond the blue expanse. As our technology continues to evolve, the prospects of potentially colonizing an exoplanet become astronomically greater, with humankind's curiosity already dabbling in the idea of commercializing space travel and colonizing neighboring planet Mars. This type of science leaves one to wonder; what if the human body could adapt to the environmental conditions of extraterrestrial planets without the need for equipment? What would it take to be capable of living a life similar to that lived on Earth but in outer space? This thesis aims to theorize what that kind of life could look like by examining the technique of bioforming the human body through directed evolution and the terraforming a planet would need to endure in order to support a functioning ecosystem and human life.

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BEYOND THE CRADLE: MECHANISMS AND APPLICATIONS TOWARDS THE COLONIZATION OF INTRAGALACTIC EXOPLANETS

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Earth is the cradle of humanity, but one cannot remain in the cradle forever.
- Konstantin Tsiolkovsky

CHAPTER ONE

A Journey Through Time: From Natural to Directed Evolution

The theory of evolution lays as a cornerstone for the essentials of life. The contributions made prior to Darwin by Al Jahiz in the 8th century, a Muslim biologist and scholar who wrote on the effect the environment had on animal's chances of survival, and by the more well-known Charles Darwin himself, as showcased in his infamous work *The Origin of Species* in 1859, have led to the expansion of the scientific world to its current and ever-growing state. Because of this, what better place to begin the voyage to space habitation than by examining what has already occurred on our own planet and delving into the niches of natural evolution.

Natural Evolution

Natural selection is the process by which living organisms adapt to their surrounding environment to increase survival odds and a reproduction rate, in most cases exponentially. This selection is typically made, by nature, as it non-randomly selects for specific advantageous traits in populations, depending on the environment and conditions (Gregory, 2009). Organisms evolve for their time and place and contribute to diversification based on these conditions. The basis of natural selection relies on five observations and three inferences asserted by Charles Darwin:

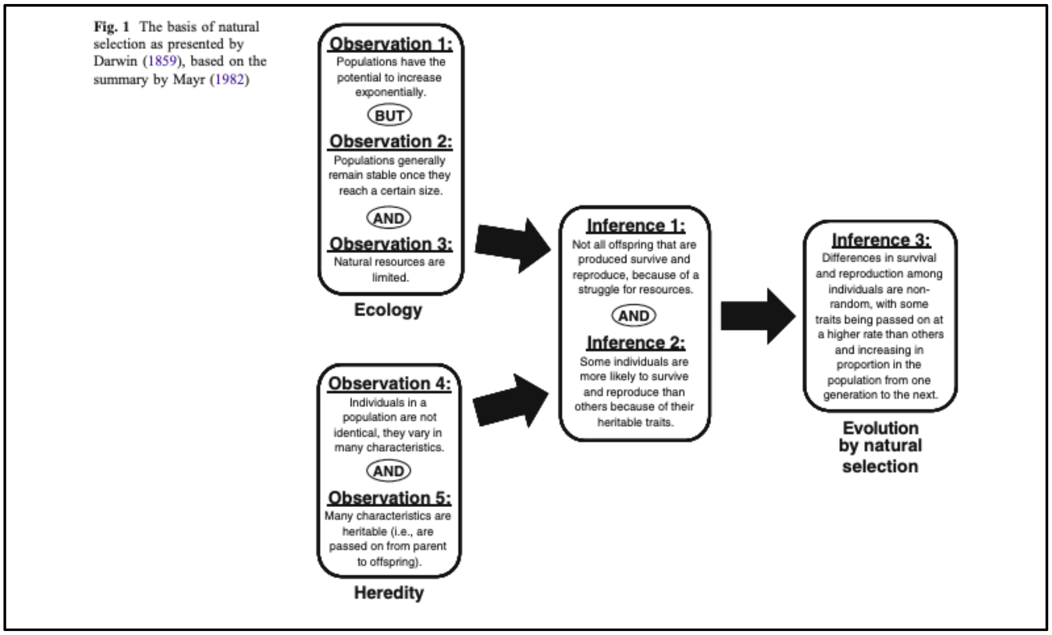

Figure 1: Darwin's observations and assertions of Natural Selection (Gregory, 2009).

Knowing the inner workings of how organisms evolve dictated by nature makes it easy to view and comprehend why and how organisms progressed into two separate groups: prokaryotes and eukaryotes.

Prokaryotes

While typically single-celled organisms, prokaryotes should not be mistaken as being simply because of this trait. These tiny creatures house all the necessary functions to produce and contribute to life within their non-membrane-bound organelles and lack of genetic storage or nucleus. They possess compact, circular chromosomes rather than linear chromosomes, which contribute to their short generation time. For example, the bacteria *Clostridium perfringens*, commonly associated with food poisoning can exhibit a generation time as short as about seven minutes (Juneja et al., 2006). This particular characteristic of prokaryotes allows for fast reproduction and low mutation rates and yet is highly adaptable, particularly to their surrounding environment. Combined, these defining characteristics help distinguish prokaryotes from their eukaryotic counterparts, allow for their unique ability to carry out the processes of transcription simultaneously, the conversion of DNA to RNA, and translation the conversion of mRNA to amino acid chains.

Eukaryotes

When thinking of life and all of the organisms that fall under the term, the first thought tends towards multicellular eukaryotic creatures, although there are some unicellular eukaryotes such as yeast. Under the group of eukaryotes falls fungi, plants, and animals, all of which possess linear chromosomes held within a membrane-bound nucleus that fills a variety of differentiated cells, each with membrane-bound organelles, that carry out various functions within the organisms being. However, due to their more complex cellular structure, the processes of replication, transcription, and translation occur more distinctly in eukaryotic cells, with more checks set in place to safeguard against possible errors and mutations and establish multiple layers of regulation.

Directed Evolution

As we have seen thus far, the idea of evolution is neither innovative nor novel. Nevertheless, it has been proven to be one of the most effective methods for accumulating biodiversity. Alas, a slight problem arises when considering the time, it takes to increase an organism's genome. The process of natural evolution has long been a task that progressed over millions of years, beginning with the Late Proterozoic to Cenozoic eras, to see its added development and progression. However, the concept of directed evolution has shifted the natural generational mechanism to a speedier, more deliberate procedure allowing for the creation of original biological processes. The fascinating aspect of directed evolution is that these invented systems can be applied to living organisms permitting previously hidden functions to be revealed, allowing organisms to obtain abilities that were once thought not possessed or possible. For example, Berman et al. discuss a method in which they hypothesize an adaptable platform for directed evolution within human cells by altering an adenovirus, removing its DNA polymerase and proteases, and injecting the resulting vector into human cells so that replication can only take place within the human cell (Berman et al., 2018). Cells altered with the adenoviral DNA would be restricted to the biomolecule of interest, meaning that a substantial amount of cells would need to be directly evolved with the adenovirus to ensure uptake and then transferred into the human cells of choice. Their platform would allow for the coupling of biomolecule activity to the altered adenovirus protease expression or activation, which would increase the genetic repertoire of the biomolecule with which it is coupled. The results could have incredible significance on the usage of biomolecular therapeutics.

Directed evolution, at its core, is a constructed procedure that manipulates biological entities, in most cases proteins or nucleic acids, and engineers them through means of random mutations with an intended goal or function by the experimenter. As with most man-made processes, directed evolution mimics its naturally occurring counterpart, natural selection. This Darwinian principle roots itself in the notion that the most advantageous or beneficial traits residing in a living system surpass and survive better than other characteristics, allowing those traits to be passed on to the offspring and their offspring. However, it is important to note that these advantageous traits are ecologically dependent. It only occurs for organisms in a particular environment at a particular point in history, i.e., the right place at the right time.

Nevertheless, a similar idea is presented for directed evolution but with more intention. The experimenter induces the natural evolutionary process to occur through the introduction of a mutation or mutations and controls the process to completion. Through the introduction of mutations, the researcher selects for a new function within a protein or the gene of their focus. The hope is that an unseen biological "key" arises, unlocking the door to an improvement from known activity or even unlocking a novel activity that will prove advantageous and thus passed on to future generations. It is important to note that if the advantageous trait is to be passed on, the parent cell's environmental factors must be kept for the daughter cells to reduce the effects of unintended mutations due to extraneous environmental factors. In summary, the process of directed evolution merely improves upon natural Darwinian evolution. Its purpose is to randomly mutate an organism so that those mutations can then be selected for as the process of natural selection emerges and takes its place.

Opening this door would allow future proteins or genes to replicate in a manner familiar to their original means of replication through natural selection, thus rendering directed evolution a more commonplace technique. Nobel Prize recipients for this technique, Frances Arnold, George Smith, and Gregory Winter described directed evolution as "…an iterative procedure which involves the identification of a starting state protein, diversification of its gene, an expression and screening strategy, re-diversification, re-screening, and so on until a satisfactory performance level in terms of enzymatic activity, binding affinity, or specificity is reached" (Linse, 2018). Paraphrasing this, the process of human-made evolution can be viewed as a series of educated trials and errors that rely heavily on the usage of "chemical intuition." This idea of chemical intuition comes as an intrinsic gut feeling, so to speak, that occurs in persons attuned to the various knowledge and mechanisms of chemical elements, molecules, compounds, and their properties.

Similar to all great scientific discoveries, directed evolution started as a hypothesis, a concept that could potentially occur if given the right tools and environment. German scientist, Manfred Eigen, along with his American protégé, William Gardiner, first presented and published a theoretical blueprint on the mechanism behind directed evolution in 1984. In their paper entitled *Evolutionary Molecular Engineering Based on RNA Replication*, they discussed how Darwin's fundamental principles focus on self-replication being the key to evolution. This process of self-replication leads to natural selection, and if this selection occurs under a controlled mutation rate, it can lead to a potential optimal biological structure. In order to achieve this, Eigen and Gardiner proposed creating and utilizing an "evolutionary machine" capable of biologically running an outlined procedure that would complete the process of evolution in vivo.

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Let us therefore expand the procedure as follows -

    10 PRODUCE A MUTANT SPECTRUM OF SELF-REPRODUCING TEMPLATES
    20 SEPARATE AND CLONE INDIVIDUAL MUTANTS
    30 AMPLIFY CLONES
    40 EXPRESS CLONES
    50 TEST FOR OPTIMAL PHENOTYPES
    60 IDENTIFY OPTIMAL GENOTYPES
    70 RETURN TO 10 WITH A SAMPLE OF OPTIMAL GENOTYPES
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Figure 2: Eigen and Gardiner's proposed step-by-step instructions on how to go about in-vivo evolution (Eigen & Gardiner, 1984)

The initial idea was not perfect, as they mentioned a potential problem that could arise with a mechanical procedure of directing the evolution process: when genotype and phenotype are not occurring within the same molecule. For example, the body can enact change on the gene expression level without expressing that alteration phenotypically, meaning the change will not be inherently visible. It is also possible that a visible phenotypic change could be expressed, but it may not be coupled with the gene in which it is typically linked. The occurrence of these potential scenarios would make it difficult to determine which modification allowed for a genotype or phenotype to arise. Despite this, Eigen and Gardiner's procedure became the catapult to launch future researchers into implementing the first usage of directed evolution within enzymes.

Keqen Chen and Frances Arnold first implemented a directed evolution procedure resembling Eigen and Gardiner's structure and techniques. Together, the two worked to find a way to custom tailor enzymatic properties by substituting amino acids within a protein sequence. For this experiment, the enzyme of choice was a serine protease known as subtilisin E. Subtilisin is a group of serine proteases derived from *Bacillus subtilis* colloquially found in laundry detergents and household cleaning products. So why choose subtilisin E for the beginning works of directed evolution? The authors suggest that serine proteases promising catalyst abilities in organic synthesis and the preparation of unique polymers naturally found that a triple variant of subtilisin allowed for enhanced enzymatic activity within dimethylformamide (DMF). They found this to be true in previous research and strived to improve enzyme activity in polar organic solvents since they typically reduce activity in these kinds of solvents despite obtaining a stable folded conformation (Chen & Arnold, 1993).

Curiosity led them to wonder what a variant of subtilisin E, composed of 10 amino acid substitutions, would have on its DMF activity. Their goal was simple: to directly evolve the protease, allowing it to function in high concentrations of DMF, a polar organic solvent. In order to accomplish this, Arnold and Chen performed a combination of site-directed and random mutagenesis, which together equate to a concept known as sequential mutagenesis. The experimenters first utilized site-directed mutagenesis to isolate the subtilisin E gene using an error-prone PCR (polymerase chain reaction) technique. The primers, oligonucleotides with the sought-after mutation, were used as mismatching primer pairs. They completed the PCR process and followed up with agarose gel electrophoresis to derive "the subtilisin gene for the 4M [mutations] variant [which] was created by adding a fourth mutation coding for the amino substitution Asp-97 à Gly (D97G) to the triple variant (3M) (D60N + Q103R + N218S)" (Chen & Arnold, 1993). The 4M variant derived from the site-directed mutagenesis was then used as the template to carry out random mutagenesis. The practice of random mutagenesis used a similar error-prone PCR method designed to increase the frequency rate of mutations after each cycle. Following the PCR cycle, plasmid construction was completed where the plasmid was placed back into the *Bacillus subtilis*, and the directly evolved bacteria were screened for increased activity in DMF. Their results demonstrated that subtilisin E variants had enhanced enzymatic activity in the polar organic solvent DMF. Furthermore, increased enzymatic activity was more effective for mutations that were located primarily around the enzyme's active site. Ultimately, Arnold and Chen's findings exemplify the idea of directed evolution proposed a decade earlier by Eigen and Gardiner and began exploring the expansion of directed evolution techniques.

Are there not others already capable of dissolving in polar organic solvents without manipulating an enzyme biologically designed by higher forces for a specific function? The answer is not a simple yes or no but rather yes and no. Sure, there are currently enzymes that dissolve perfectly fine in polar solvents and are utilized in synthesis reactions to do just that. However, while they use the same set of amino acids, each protein differs in its deliberately formed sequence. The sequence or arrangement of those amino acids is what determines their functionality. By substituting a single amino acid without altering the rest of the series, as seen in the experiments performed by Chen and Arnold, the majority of the enzyme's integrity is functionally the same except for the single substitution point that in the example explained above allowed for it to be soluble in solvents not previous soluble to that enzyme. By keeping a majority of the enzyme's functionality, the enzyme can now perform functions it used to perform elsewhere, in a new area which then gives rise to neoteric roles such as the variant subtilisin's ability to "acylate sugars and steroids regioselectivity and will catalyze peptide synthesis either by direct reversal of the hydrolytic process of by aminolysis of N-protected amino acid or peptide ester" (Chen & Arnold, 1993). Stated simply, prior to the direct evolution, subtilisin had the potential for enhanced activity within polar solvents such as DMF.

After the procedure, subtilisin's potential was unlocked and revealed its ability to introduce acyl groups based on regioselection and catalysis of peptides.

The work done by Arnold and Chen led others to refine the techniques used to undergo directed evolution. However, a consistent problem that arose was the idea of trying to insert changes irregularly throughout the entirety of an enzyme or gene. The number of mathematical combinations that could occur would be impractical and inefficient to pursue in a lab setting. When Dutch scientist William Stemmer brought forth his idea of "DNA shuffling," a technique that allowed for the recombination and propagation of advantageous mutations, it further propelled the usage of directed evolution. DNA shuffling provides the "recombination of DNA from similar genes from several organisms, [which] introduces more variation than many other methods and can thus improve the chances to reach a substantial activity increase in the evolved variants" (Linse, 2018). Performed in vitro, there is homologous recombination of pools from selected genes through the process of polymerase chain reaction. Stemmer found through experimentation that a more useful approach to DNA shuffling may be to “shuffle many related, naturally occurring genes, such as antibodies or homologous genes from different species” (Stemmer, 1994). Suggesting that the diversity created in this means may be more meaningful that if solely relying on random mutations. Stemmer's contribution to DNA shuffling allows for efficient evolution and the expansion of the DNA library.

Others soon began to add to the skeletal structure of directed evolution and began fine-tuning its methods and uncovering pieces to the procedure that they did not realize were missing or needed. Dan Tawfik, a biochemist at the Centre for Protein Engineering and Laboratory of Molecular Biology in Cambridge, demonstrated that directed evolution did not need to be jumpstarted with living cells but could be performed in synthetic cells, a growing field is known as synthetic biology. Cellular compartmentalization is the cell's natural way of linking the phenotype and the genotype by sectioning off the genes, proteins, and nucleic acids that they encode with the product that those components form. Tawfik, along with his partner Andrew Griffiths, discovered that the process of compartmentalization could be performed in a synthetic cellular construct, allowing for directed evolution to be performed within the "cell" chamber and for the phenotype and genotype of the changed variants to be kept together and more easily distinguished. They call their human-made compartmentalization technique "Genesics" (Gene Selection in a Compartmentalized In Vitro System). Tawfik and Griffiths demonstrated their idea by using water-in-oil emulsions acting as aqueous compartments. Specifically, they focused on methylated DNA to prove their system because it proved that the most beneficial aspect of a system such as theirs is "the ability to select catalytic proteins directly by product formation" (Tawfik & Griffiths, 1998). Even more intriguing Tawfik and Griffiths found that a key advantage to compartmentalization is that it can potentially allow the selection of catalysts that reacts with different substrates that are not attached to the genes, the benefits of which combat the previous limitation of substrates being unable to be attached and retain their functionality. The idea of gene selection through compartmentalization brought on another nuanced feature in the directed evolution process, as now not only could directed evolution be performed with a synthetic controlled environment but the selection within gene libraries to create the variants typically sought after was now slightly more simplified.

Currently, the most common technique utilized for directed evolution is not a technique at all but rather a set of guidelines, as the directed evolutionary process cannot be replicated the same way for all desired biological variants. As mentioned prior, it is a series of educated guesses supplemented with knowledge of the biological entities to allow for a theoretical blueprint. Nevertheless, the guidelines and the aforementioned eureka moments found while unearthing the mechanisms of directed evolution have provided a stable backbone for researchers to expand upon. One guideline of importance is: if the starting entity of the directed evolution process has a natural function of high activity, it is best to lower the natural activity first before proceeding to evolve it directly (Linse, 2018). By not lowering the more favorable activity first, the goal function trying to be achieved will be extremely difficult and less likely as it is competing against the natural activity versus a non-natural activity. In other words, it reduces the ability to branch off to other potentially better possibilities. The effort and energy needed to overcome the natural process would be similar to asking Han Solo, the infamous intergalactic smuggler in the Star Wars Universe, not to fly the Millennium Falcon or have an enzyme complete a reaction without a catalyst.

While the information presented within this chapter is not by any means a comprehensive list of the achievements and people whose efforts have brought forth the revolutionary idea of directed evolution, those mentioned have propelled it to the studied scientific mechanism that it is today. Directed evolution has been used and is currently being used to access new possibilities in the realms of biofuels, new chemical bonds, environmentalism, pharmaceutical medicine, and the list goes on. What if this accelerated evolution could be used within the human body? Imagine if this technology could be applied with the hopes of propelling humanity into inhabiting and potentially colonizing a planet in space?

CHAPTER TWO

If Terraforming was a Mutually Exclusive Event

In the 1960s, the US entered a space campaign to land the first man on the moon. Undoubtedly, the success of the moon landing and the infamous words of Neil Armstrong, "One small step for a man, one giant leap for mankind," have spurred the world towards greater space exploration. Ambition has grown, and goals have become more extravagant as space explorations have entered the private sector. Billionaires, along with NASA, have their sights on the big red planet of Mars. The goal is not just to set foot on the neighboring planet but to be able to inhabit it. For some, this possibility is seen as a viable backup plan for the inevitable downfall of Earth due to the growing global environmental crisis. In contrast, for others, this is a promising vacation location. However, in its current state, Mars is unhabitable to living organisms without the protection of a spacesuit or greenhouse buildings that keep out the unfavorable conditions of the novel planet. While habitation could feasibly occur in the way described above, is it sustainable? Is life in suits and space pod-like buildings a life that wants to be lived? What if Mars itself could be evolved and made habitable so that the transition from Earth to the Red Planet is practically seamless?

Before excitement builds as to the possibility of potentially modifying Mars, it would be good to understand terraforming and the variety of forms it can take, depending on the desired result. The word terraforming means "Earth-forming," to model a planet in Earth's likeness. Just as there is no one way in which a tub of Legos can be put together, there are different potential approaches to the act of terraforming a planet, and these approaches are goal dependent.

Suppose the goal is to allow a civilization to be self-sustaining. In that case, the approach may resemble a civilization living in suits and greenhouse buildings, similar to those seen in Frank Herbert's *Dune*, to protect from the unaccommodating environment of the red dust storms and lack of oxygen on Mars. The downfall to this approach is despite the aid of technology the toll of living in space will eventually have a tremendous effect on the human body, leading to deterioration of functions and abilities. Nonetheless, the civilization could be self-sustaining as they are able to live and sustain themselves with the help of technology built to allow them to do so.

If the goal is to allow humans to live healthy lives as in preventing the negative effects of living in space from occurring, then terraforming would take the form of looking into the effects of gravity, radiation/cosmic rays, perchlorates, and toxins on the human body. In this case, terraforming would result in alterations that adjust the atmosphere to protect it from radiation or tinkering with the planet's overall mass to, in the case of Mars, increase gravity so that it resembles gravitational forces found on Earth.

If the goal is to make the planet habitable for microorganisms, then terraforming, in this case, would be the most straightforward option as one would simply take microorganisms, such as extremophiles and tardigrades, that can survive in the most extreme of environments and perhaps make the slightest changes to the planetary environment such as ensuring there are the proper nutrient and energy sources that will allow them to replicate, which would constitute life.

Lastly, if the goal is to allow humans to survive without technological support systems, which is the slightly outlandish yet optimistic and whimsical approach this thesis hopes to delve deeper into, what would terraforming look like then? Ideally, this is the preferred goal, at least in this author's opinion. To better understand what this last form of terraforming might look like, it would be best to look at a planet on which humans are already surviving without needing technological support systems: Earth. What makes Earth special?

Water

A myriad of distinct factors come to mind when one thinks of the unique factors of Earth. However, the most immediate and salient feature is water, specifically liquid water. The importance of water extends beyond the human form. While water makes up about 75% of the human body and humans can only withstand approximately three days without it, water has implications for all forms of life, especially when one begins to look at the cellular level of living organisms. Water is composed of two hydrogens atoms and a single oxygen atom that compose a molecule with a unique tetrahedral bent configuration.

The configuration of water allows for it to display a special type of bonding due to the polarity exhibited by the more negatively charged oxygen atom and the partial positive nature of the hydrogen atoms. The bonding is known as hydrogen bonding and typically occurs between other water molecules as well as molecules with free nitrogen, oxygen, sulfur, and or fluorine atom attached. This unique ability underlies water's unique properties, including the vast difference between its freezing point, 0 degrees Celsius, and its boiling point, 100 degrees Celsius. Due to this difference, water can remain a liquid over an extensive range of temperatures, explaining the phenomenon of water within lakes remaining fluid despite an external below freezing temperature is below freezing and the lake's surface being frozen solid.

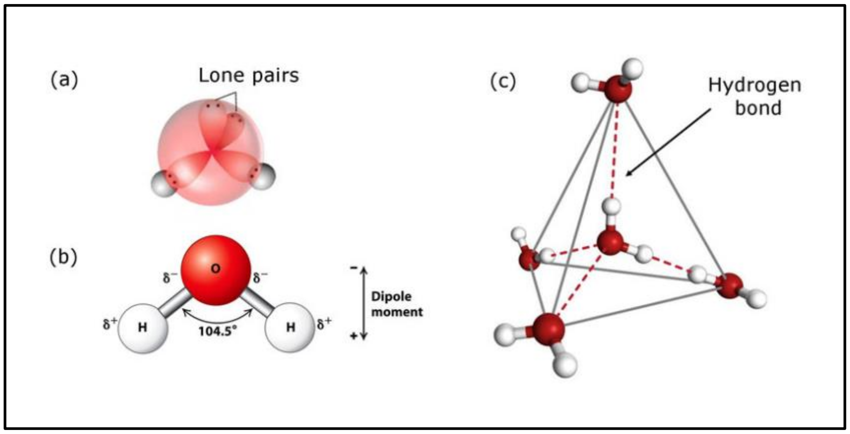

Figure 3: (a) The electronic distribution in a single water molecule, red being oxygen atom and the white being the two hydrogen atoms. (b) The tetrahedral structure and bond angle formation of a water molecule which aid in creating the polarity of the molecule. (c) The arrangement of multiple water molecules to form hydrogen bonds (Stefanutti, 2019).

Even more exciting, hydrogen bonding takes effect in the macromolecules of breathing organisms. For example, suppose one looks at the structure of DNA. In that case, one will see that the double-helical nature of the structure is due to the unequal hydrogen bonding between the paired nucleotides, A-T (two hydrogen bonds) and G-C (three hydrogen bonds). Structure, more often than not, is related to function. Therefore, the role that hydrogen bonding plays in maintaining the structure of DNA allows for DNA to continue functioning as the blueprint for cell functions and organism traits. Hydrogen bonding works similarly in proteins and polysaccharides as well.

In proteins, hydrogen bonding acts in the secondary structure, where alpha-helices and beta-pleated sheets fold. Correct folding ensures the correct form of the protein arises, leading to the correct biological processes, which ensures the efficient functioning of the organism. An example of the immense effects of protein misfolding can be seen in Creutzfeldt-Jakob disease. In Creutzfeldt-Jakob disease (CJD), there is a buildup of prions. These misfolded proteins accumulate and have the ability to cause other proteins around them to become misfolded, specifically within the brain. This accumulation of misfolded proteins leads small holes to develop, transforming the once compact matter of the brain into a sponge-like essence and leading to the development of plaque aggregates similar to those seen in diseases such as Alzheimer's and Parkinson's. These confounding effects lead to the neurodegenerative effects of CJD, such as progressive dementia and myoclonus. In carbohydrates, hydrogen bonding works to create stable polysaccharides for energy storage, such as glucose and cellular communication by being covalently bonded to a lipid or protein. Water is required for proper cell functioning, and without it, life struggles to exist, mainly by facilitating chemical reactions and bonding. As a result, Earth harbors a great variety of life compared to other planetary bodies, and this explains why Mars being devoid of it makes it all the more difficult for life to exist on the Red Planet.

Before moving forward in the discussion, it should be noted that while water is an essential factor for all living life, it is not the sole reason why Earth harbors life. Ocean worlds such as Europa, Titan, Pluto, Triton, and Mimas, to name a few, all possess some form of water and yet do not contain life, at least life that is yet known. A myriad of combined factors contributes to habitability, and therefore the assumption that if an exoplanet has water, it can be lived upon should not be accepted wholly.

Magnetic Field

In the case of water, the challenge, while challenging, does not feel entirely implausible or intangible. By adding water, one is adding a substance that may not already exist on the planet of choice but can potentially be created and manufactured on the planet through scientific means. However, in the case of changing atmosphere or the magnetic field, the challenge becomes considerably more daunting. How does one change the force that a planet produces? Well, fear not because it might be more feasible than it appears!

Without getting too into the physics behind a magnetic field, a magnetic field is "an invisible field that exerts magnetic force on substances that are sensitive to magnetism" (*What Is a Magnetic Field? - Universe Today*, n.d.). Applying this definition to a planet, it is seen that the magnetic force is generated from the electrically charged core held within a planetary body. It is the churning the movement of this core that causes the magnetic field or magnetosphere (*Earth's Magnetosphere | NASA*, n.d.) of a planet to generate outward towards the planet's surface and engulf it creating a sort of invisible magnetic shield that in the case of Earth protects it from the solar winds produced by the Sun. All planets have a magnetosphere; what distinguishes them from one another is their strength and ability to protect them from the celestial body around which they orbit. The strength of its magnetic field is why Earth is unique; it is another of many reasons why Earth harbors life.

Further investigation into Earth's magnetosphere showcases that it is primarily composed of iron in different forms: a molten form and a more solid form. Studies suggest that the molten form of iron is held in the majority in the outer core of the life inhabiting planet, where it, along with some lighter elements, flows to create a current. This movement creates the magnetism felt by Earth. David Waltham explains that "this motion [the current formed from the molten metal core] is assumed to consist of convection currents driven by cooling of the inner core and by compositional buoyancy produced as light elements are expelled from freezing iron at the surface of the solid inner core" (Waltham, 2019). The movement of the molten core that generates the currents discussed is known as geodynamo, which for Earth, is uniquely global in nature, meaning that the magnetic field encompasses the entire planet. This differs significantly from Mars (Steigerwald, 2020).

Instead of having a global magnetosphere, Mars has an induced one. For Earth, the magnetism produced by the planet comes internally from currents within the core. However, in the case of Mars, the magnetosphere is produced externally from the upper atmosphere that is ionized by solar radiation, which strips the electrons from atoms in the atmosphere, creating a plasma state. This state of plasma is considered extremely conductive. It allows for electric currents to flow through it, similar to the electric currents found within the Earth's core but are instead found externally. The extent of what is known about the nature of Mar's magnetosphere is limited. However, currently, NASA is determining its nature and the potential for it to be changed using the MAVEN spacecraft launched back in 2013. From the information gathered thus far, researchers have found that Mar's atmosphere used to be thick and full of carbon dioxide, molecular nitrogen, and argon, perhaps even fuller than Earth's, which helped maintain a warm and wet environment in its former life. Over time, due to the nature of its magnetosphere being external, the toll of solar winds rushing up against the planet and wrapping around it, plus intermingling with its external electrical currents allowing for a stronger electrical connection, analogous to winding yarn on itself to create a growing yarn ball. The "yarn wrapping" occurs due to the fact that the solar winds carry their own electrons and ions, which oppose the electric charge of the induced magnetic field of Mars. This causes some ions to flow in one direction and others in another, which creates the wrapping effect. However, the currents from the solar winds and induced magnetosphere of Mars drive some of the magnetic and electrical fields generated to push charged atmospheric particles outward into space. Atmospheric particles are typically lost to space, but this process became expedited with the aid of the magnetic and electrical fields. This loss of atmospheric particles led to a deterioration in the planet's atmosphere, known as an atmospheric escape, which helps to explain why the Red Planet went from wet and warm to dry and cold. The loss of Mar's atmosphere highlights the importance of magnetic fields and their impact on a planet's atmosphere that, relates to the planet's environment.

The Atmosphere and Atmospheric Pressure

Understanding that a planet's magnetic field has a tremendous influence on the planet's atmosphere is noteworthy, although not all-encompassing, of the aspects needed for a planet to have an atmosphere conducive to hosting living organisms. Having what is deemed a good atmosphere is vital to sustaining life on a planet. A good atmosphere can be defined based on the atmosphere found on Earth due to the fact that it does the following: Acts as a protective blanket that traps heat, keeping the planet's temperature at livable conditions; Shields the surface from harmful radiation exerted by the star in which the planet orbits; And provides essential elements needed for life such as nitrogen, carbon dioxide, and oxygen.

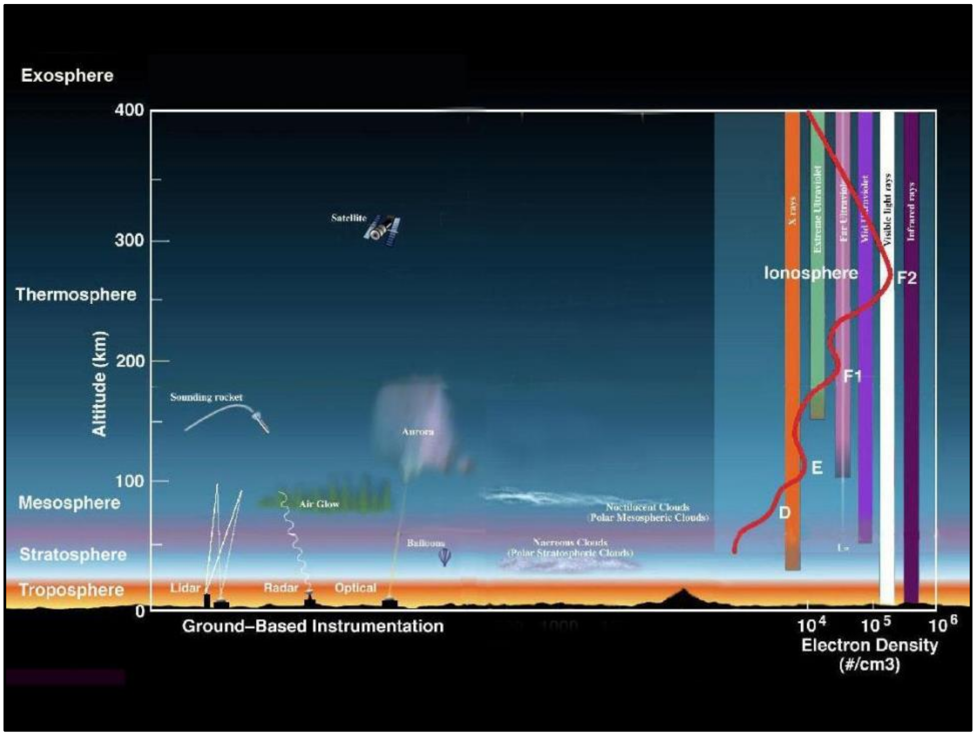

Figure 4: Layers of Earth’s Atmosphere
(*Earth’s Atmosphere*, n.d.)

Earth's atmospheres consist of five layers: the outermost layer called the exosphere, followed by the thermosphere, the mesosphere, the stratosphere, and the troposphere is the innermost layer and the most familiar to life on Earth. A sixth layer known as the ionosphere is perpendicularly spans the mesosphere, thermosphere, and exosphere and interacts with the magnetosphere mentioned in the magnetic field section above. The focus of a habitable planet lies in the troposphere because of one essential feature: this layer possesses the air that all living organisms breathe or utilize. The troposphere is defined as the shallow layer of the atmosphere that extends about 12 kilometers from the Earth's surface. Within, this layer is a measured mixture of gases heavily composed of nitrogen and oxygen with traces of argon, hydrogen ozone, and other more minor, less significant constituents (*Earth's Atmosphere*, n.d.). Interestingly, the troposphere also contains about 99% of all the water vapor and aerosols needed, needed by life, which helps in its contribution to the seasonal weather typically enjoyed on planet Earth. Most significantly, though, is that due to the nature of the troposphere being 78% nitrogen and 21% oxygen, it contains the needed material for plants to photosynthesize and animals to breathe. Understanding the composition of the troposphere allows for the understanding that all living things need an atmosphere that contains a significant amount of nitrogen and a comparable amount of oxygen in order to survive. While it is unsure how many layers of the atmosphere the Red planet has encompassing it, what is known is that Mars' atmosphere is almost entirely composed of carbon dioxide (95%), with the remaining five percent being made up of molecular nitrogen (2.6%), argon (1.9%), molecular oxygen (0.16%), and carbon monoxide (0.06%) (*Curiosity Rover Serves Scientists a New Mystery: Oxygen | NASA*, n.d.). Creating a conducive extraterrestrial environment for life requires the alterations of the composition of Mars' atmosphere.

Another aspect of Earth's atmosphere to consider adopting to an inhabitable planet such as Mars includes Earth's unique ability to cleanse itself. Within the many layers of the atmosphere are hydroxyl radicals [-OH], that float about and clean the atmospheric air from pollutants, specifically ensuring that the concentrations of carbon monoxide, sulfur dioxide, hydrogen sulfide, and methane remain at sustainable, nonharmful levels (Laboratory, n.d.). By having this additional layer of protection within the atmosphere, Earth has an internal alarm of sorts that constantly ensures that the atmosphere maintains the delicate balance of elements needed for its continued usability for organisms inhabiting the planet. Including a system like this on Mars could contribute to a more stable atmosphere once the composition of the said atmosphere has been altered to harbor life. Overall, Earth's unique atmosphere, with its six interacting layers and unique elemental composition, provides insight on the nuances needed for life to form and continue living in their environments.

Temperature

As mentioned above, Earth's atmosphere exhibits a more consistent temperature and consistent seasons that life on the planet has adapted to and continues to permit life. A consistent and moderate temperature, as opposed to extreme temperatures (too hot or too cold), allows for less stress to be placed on the life systems and influences the rate and speed at which molecules move. More fluid and rapid movement of molecules allow specific biological functions such as metabolism to continue functioning under homeostatic conditions. This idea can be demonstrated with a cockroach and a SpikerBox, a device used to measure neuronal electrical activity!

Generally, most electrical signals are sent via neurons in the central and peripheral nervous systems through action potentials. Depending on the temperature, the firing rate at which the action potential is released and subsequent axonal signals sent can vary wildly. In the SpikerBox experiment, a cockroach was placed in ice-cold water, inducing hibernation. As the temperature drops, neurons in the cockroach leg become inactive as ion channels (Na^+, K^+) decrease in the release of ions, making the creation of an action potential increasingly tricky. As the temperature drops below the "threshold" temperature that allows them to continue functioning, the ion channels lose all ability to open, thus preventing the efflux or influx of ions resulting in the loss of the action potential. Essentially, the amplitude decreases gradually, with a decrease in firing potential until all of the ion channels are no longer functional, preventing the cockroach from moving.

At high temperatures within the same SpikerBox experiment, the cockroach was placed under a candlelight flame. There was a gradual increase in action potential firing as the temperature rose. During this rise, the ion channels release their ionic contents into and out of the cell. However, when the temperature became too hot, a "threshold" was met, resulting in the ion channels having discharged the entirety of their contents. At this point, the firing ceases… forever.

Several significant takeaways from the SpikerBox experiment apply to the capability of living on intragalactic planets. When the temperature reaches extreme cold and chill, the neurons are not permanently dead but rather in a stasis mode. The neurons adopt a state of "hibernation" and are able to "defrost" upon being placed in a room-temperature environment. However, as seen with the hot temperature effects on neurons, the neurons reach maximal exertion and ultimately die. The ion channels expelled all their contents and could no longer produce an action potential since there are no ions to exchange between the membrane, and the neuron is dead. Even once temperatures are restored to "normal" (room temperature, 25OC) conditions, the neurons retain their lost ability to fire again. These findings translate to other eukaryotes, specifically animals and, even more specifically, humans.

Other factors to consider when discussing temperature include the planet's distance from the star in which it orbits. For a planet like Mars, it is understood that it is further from the Sun than Earth, resulting in colder temperatures. Of course, moving the Red Planet closer to the Sun would be rather implausible, but understanding the importance of temperature informs the terraforming process to create more reliant and ideal temperatures. Without the "just right" temperature, as Goldilocks might say, cultivating an ecosystem in space will be an even more remarkable feat than initially expected.

Energy and Nutrients

In general, when looking into planet habitability, researchers look for a particular set of nutritional biomarkers that potentially increase the odds of the planet harboring life or potentially being able to harbor life. According to Mudhusudhan et al., reliable biomarkers, mainly found within the atmosphere of the observed planet, that should be considered noteworthy includes elements such as O2, O3, CH4, and N2O, all of which are found within Earth's atmosphere. Others of importance originated from metabolic processes found on Earth, including organosulfur compounds, such as methanethiol (CH3SH) and dimethyl sulfide (DMS), to name a few.

The importance of these specific elements to producing a stable ecosystem is that they all contribute to the providing the necessary energy for the metabolic processes of living organism as well as are prevalent in the composition of these organism’s physical structures. To add to this, having the right elemental components that compose the available energy and nutrient availability of a planet are important from determining energy flow in the ecosystem that in turn ensure a healthy ecological balance.

All these factors make Earth unique in its ability to harbor life, not just human life as we know it. This is a rather vital notion to consider when discussing the terraforming of Mars. Humans' colonization of the Red Planet must also have all the other variables contributing to their existence, such as the human relationship with plants and their exchange of CO_2 for O_2. All living systems live in a schema of each other, each having its own abilities and functions that contribute to the overall ecosystem. If humans are to live on Mars, then the ecosystem of Mars needs to be altered so that humans, along with other living organisms that are not typical of the planet, can begin to contribute in some way to the created environment. The question now is, how can this be done? Terraforming is not one particular technique but rather an amalgamation of techniques, each utilized for its advantages and ability to combat the disadvantages of other techniques. The whole process is rather complex, layered, and nuanced. Terraforming to have humans survive without reliance upon technological support systems requires initial mechanical engineering of the planetary body.

Terraforming Techniques

In the Marvel TV series *What If*, season 1 episode 2, the characters discussed a nutrient-rich cosmic dust from an ancient supernova, known as 'Embers of Genesis', that has the power to terraform entire ecosystems. While, possessing a cosmic dust of this nature would make the entire terraforming process much easier, it unfortunately does not exist in this timeline. However, there is something of a similar nature that can be utilized to perform similar functions to the Embers of Genesis, not dust but machines. The usage of mechanical engineering.

To begin terraforming, the necessities of a planet must be met first, which were previously discussed in comparison to Earth's factors. Many of those factors, require physical, structural changes to the planet that will be difficult produce without some form of mechanical engineering. Thus, to jumpstart the process of terraforming, one needs to mechanically engineering the planet first. While, this type of terraforming is beyond the scope of the thesis and authors expertise, it should be acknowledged as the first approach before any of the other concepts that will be mentioned in the section are considered and utilized.

One thing to considering, when attempting to create a functioning ecosystem is that other life, other living organisms besides humans, reside and are integral "cogs" in the human schema as well as the ecosystem itself. A classic example of this would be plants, providing oxygen for humans to breathe as it being an expelled byproduct in their metabolic cycle of photosynthesis. With this, it leads to a potential idea, where once mechanical engineering has been used, the planet can be seeded with microbes and microorganisms such as extremophiles and cyanobacteria, that can be used to begin conditioning the planet, similar to what is known about early Earth conditions. In fact, the use of directed evolution, could come into play with the method of seeding as, it would be possible to directly evolve these microbes with specific functions, such as producing and expelling some of the key nutritional elements discussed into the planet's atmosphere, as byproducts of their own metabolic functions. In this way the organism begins to prepare the planet's environment for more complex life.

However, a potential draw back with seeding a planet would be that in order to get those tiny organisms on to the planet to perform the preparatory procedures for habitability, it would need to be primed for them, just as they are priming the environment for other life forms. Meaning, for example, if cyanobacteria were to be used as primary seeding agent, questions to think about would be what they need to survive and is it already available on the planet. If it is not already available, who then does one go about making it readily available so that they can thrive and in turn prime.

Another potential idea would be to let the galaxy run its own course and potentially terraform the planet itself. What is meant by this? In Spina et al., the authors performed statistical studies which led them to find that there is chemical evidence for the planetary ingestion of Sun-like stars. Essentially, what they found was as an evolutionary mechanism, the stars, similar to that of the Sun, slowly engulf fractions of the their orbiting planets chemical composition, creating an adapted composition for itself and altering the composition of the revolving planet (Spina, 2021). It still unclear, of the exact process by which this engulfment proceeds but it leads to the thought that potentially, with the star and planet both changing their makeup, while random, this sort of change could bring about the necessary conditions that could host an ecosystem.

Throughout this chapter, various forms of terraforming the red planet were explored, emphasizing the use of directed evolution to do so. However, ultimately, once this goal is accomplished, and Mars has been terraformed to resemble its neighboring life-bearing planet, would it still be Mars? By terraforming Mars alone, meaning not altering the physical forms of the humans and varying life forms that will soon inhabit it, would we then not just be creating an Earth 2.0? The essence and the novelty of Mars would get swept up in trying to make it habitable, which in turn defeats the purpose of extraterrestrial living. If humans want to live on Earth, they are more than welcome to remain Earthbound. However, for the adventure-filled and curious humans, living on Mars is more of a longing to explore different terrains and environments; they live for the novelty of space exploration. What fun would that be if Mars became another Earth? Therefore, if we are unwilling to change the form of Mars, then the human form itself must change.

CHAPTER THREE

If Bioforming was a Mutually Exclusive Event

When a person thinks about the meaning of bioforming, the definition "forming life" becomes apparent by simply parsing the word. More specifically, bioforming is the notion of taking biologically living organisms and altering their existing capabilities to improve. This "improvement" can present itself in a number of different ways, including increasing the efficiency of the organism's constitution or perhaps in the enhancement of natural characteristics and mechanisms such as taking Vitamin A supplements to maintain normal vision and immunity. However, this can lead to ethical issues like what constitutes a beneficial change, which will be explored in a later section. Nevertheless, this idea of bioforming shares an association with science fiction and the fantastical world of the imaginary, thus causing many to believe bioforming to be a technique of hypotheticals and nonexistent.

Quite the contrary. The psychostimulant methylphenidate, colloquially known as Ritalin, is used to treat attention deficit hyperactivity disorder (ADHD). It works by binding to a dopamine transporter or norepinephrine on the membrane of a dopaminergic or noradrenergic neuron, which blocks the reuptake of dopamine and norepinephrine. This blockage causes the elevation of the extracellular levels of these neurotransmitters and, due to this increase, stimulates the overall catecholaminergic neurotransmission providing an optimal amount of dopamine and norepinephrine to reach and activate their respective receptors, thus providing increased alertness and concentration. Ritalin's action on the nervous system is bioforming. It takes the individual who struggles to have singular focus and control impulses and redirects the body through chemical changes to allow them to become single-minded in attention. That is to say, for Ritalin to have an effect, it must bioform; in the same way, much of modern medicine relies on this concept. Bioforming has been and is amid daily life and routine. For example, drinking a morning cup of coffee is bioforming. The ingestion of black bean water allows caffeine, a chemical compound found within the coffee, to act as an adenosine antagonist. This neurotransmitter inhibits neuronal excitability by decreasing the release of most neurotransmitters in the brain. Because of this, adenosine works to reduce arousal, increase sleep, and suppress spontaneous behavioral activity (Davis et al., 2002). Caffeine's ability to block adenosine by binding to adenosine receptors provides temporary relief by delaying the effects of fatigue and exhaustion, which increases alertness and wakefulness, which have proven beneficial to many students, professors, and persons within the workforce.

However, there is always a time limit or duration that is eventually met before secondary adjustments need to be made or another dose needs to be administered. The goal of bioforming under directed evolution would be to take what has been temporary and increase its sustainability by allowing the body's natural mechanisms to take over or even go against the innate mechanisms to correct a detrimental mutation. Ultimately whatever characteristics are altered employing directed evolution, the hope is that the once novel traits become selected for and are then considered advantageous. Through bioforming, a more permanent faster evolutionary road is available to fulfill the plots of a myriad of science fiction novels and movies.

Living on Mars might be closer to reality if all one needed to do was alter their physical being without infringing on the environmental integrity of the planet. What would it look like to change the human form without utilizing terraforming, making it habitable on Mars? There are multiple ways this could be theoretically done, some of which will be explored within this chapter. Before one gets to this point, it helps to first look at how humans naturally perform their existing functions through good old Darwinian evolution.

Modern humans are mammalian creatures composed of approximately 75 trillion eukaryotic cells. Each cell within the human has fine-tuned functions that work together to complete processes of which the person is completely unaware. For instance, astrocytes, glial cells that act within the central nervous system to improve synaptic activity, increase neurotransmitter uptake, keep up with metabolic demands, and increase blood flow to improve glucose and oxygen delivery, to name a few. Interestingly, the unique size and complexity of these glial cells in humans aid in increasing their "functional competence" in humans' brains, which Oberheim et al. suggests bringing rise to humans distinct neurological capabilities such as social cognitive skills. Other cells like pancreatic cells, as one example, secrete hormones such as insulin and glucagon to control plasma glucose concentrations within the body, all of which are controlled autonomically by the nervous system but are not uniquely human as sharks and others also possess pancreatic cells that help them to control their metabolisms (Oberheim et al., 2009). This is rather noteworthy because it showcases the unpredictability of divergent evolution or the evolution by which species share a common ancestry but become more distinct due to differential selection pressures, leading to speciation. For example, humans and sharks contain the same cells with similar functions, but the two species could not look and be any different (Gautam, 2020). It is for this reason that the use of directed evolution to enable humans the capability to live on Mars or any other planet capable of harboring life would be more beneficial as directed evolution is more focused and nuanced; the worry of humans diverging into other groups can be controlled utilizing this technique.

How did natural evolution bring about humans, and how can that process help focus efforts on evolving humans without the concerns of possible divergence? The humans of now came about through the infamous six stages of human evolution: *Dryopithecus, Ramapithecus, Australopithecus, Homo erectus, Homo sapiens neanderthalensis, and Homo sapiens sapiens*.

The first of the six stages, *Dryopithecus*, connects humans with their primate counterparts the apes. They were most likely herbivores and lived-in modern-day China, Africa, Europe, and India.

From there, evolution spawned the *Ramapithecus,* where they sported a similar strong jaw structure that humans possess today, tooth enamel, and the usage of their hands for food and defense. This concept of their hands being used for food and defense led to the notion that they could have potentially been creatures who obtained an upright posture.

Following, Ramapithecus was the *Australopithecus*, who, based on fossils found in South Africa, walked definitively upright and utilized objects in their environment as weapons.

From Australopithecus, the infamous *Homo erectus* came into existence, sporting the more prominent cranial features. The larger head led to the notion of greater cognitive abilities, which correlated to their creation of fire, the use of bones, wood, and quartz for tools/weapons, and group collaboration.

The appearance of *Homo sapiens neanderthalensis* led to the modification of several of the features listed in the previous evolutionary group. For example, the head size grew even larger! They were also discovered to have used hand axes as well as began hunting animals greater in size, such as the wooly mammoth.

Lastly, the most recent and the current evolution of human beings is the *Homo sapiens sapiens*. The size of cranial features was reduced from its previous bulbous size to a smaller, more rounded shape. In addition, the appearance of the "modern chin/jaw" was highlighted in this group. Although the head size was reduced, cognitive abilities continued to grow as the discovery of man-made art was found and the concept of group hunting for food became more sophisticated.

Through each stage that humans have moved through in order to become who they are in this current age, slight modifications emerged, prevailing as the more advantageous trait and establishing a new standard of "human." As a species, natural selection was followed, just as Al-Jahiz and Charles Darwin predicted. In fact, *Homo sapiens sapiens* is not even the last stage of the human evolutionary process as humans are currently still evolving. Nevertheless, suppose the current human beings of the world want to be able to explore exoplanetary living. In that case, the evolutionary transition from *Homo sapien sapiens* to whatever is next, perhaps *Homo superiors*, as suggested by X-Men villain Magneto, must be dictated not by nature but rather by people themselves.

While the corporeal body of humans is evolving in small yet significant ways by nature's whim, we now possess techniques such as directed evolution that could allow humans to take the process of evolution into their own hands. Because of these newfound capabilities, it might be helpful to take a more microscopic view of the human body, delving into the eukaryotic cells that compose it.

The rise of eukaryotic cells has always been a source of mystery. Currently, the most accepted model, proposed around 1905 by Russian botanist Konstantin Mereschkowski, that is evidenced is the endosymbiotic theory. The theory details the divergence of prokaryotes into eukaryotes through a symbiotic relationship between alpha-proteobacteria/cyanobacteria and the host cell. This process is thought to have occurred roughly one and half billion years ago through the phagocytosis of mitochondria/chloroplast that used to exist as independent entities. The evidence for this theory can be seen in the unique traits held solely by mitochondria and chloroplast, each of which contains their own DNA distinct from the organism's DNA and the fact that they can divide through simple fission, a process independent of the cell cycle. Curiously, other theories beyond endosymbiosis have arisen as well.

Moreira and Garcia propose a novel hypothesis that there is a theory in contention with the currently accepted belief of eukaryotic emergence: the syntropy symbiosis. In this theory, eukaryotes spawned through the rise of metabolism symbiosis of a prokaryotic bacterial cell and methanogens. The process required that the bacterial cell engages in fermentation, leaving as a byproduct H2 (hydrogen) interspecies, which methanogens would then take the H2 and convert it into methane (Moreira & Lopez-Garcia, 1998).

No matter how eukaryotes truly evolved, one factor remains constant in the two proposed theories: Symbiosis.

Symbiosis played a significant role in the birth of eukaryotic cells, but it has now become more apparent that symbiosis is in constant flux in the biological world. This idea is made clear prior to the eukaryote's humble beginnings theories. In the realm of science, all biological organisms interact in some form or fashion to achieve their required functions. This need, explicitly focusing on the interaction between tissues and organs within a single organism, to be able to work with other units of life to complete more precise functions, leads one to theorize that perhaps the way to bring about novel advantageous functions could be done by using directed evolution to create that symbiosis. To do this, several different concepts come to mind.

The first idea involves directly evolving microbes and placing them within the body to create a syntropy soup of sorts. The idea would function similarly to the proposed eukaryotic theory of metabolic symbiosis mentioned earlier. For example, the gut microbiome, or the bacterial environment that resides within the human gastral/intestinal system, has a special interaction with the brain where the gut bacteria stimulate afferent neurons via the vagus nerve of the liver. These signals inform the central nervous system of ingestive behaviors such as hunger and thirst and influence human sleep patterns and stress reactivity (Galland, 2014). Suppose perhaps certain functions could be gained from other organisms residing within the human body, similar to the gut microbiome and brain interaction. In that case, one example may be humans potentially gaining a new respiration pathway that differs from the two currently used, aerobic and anaerobic. Aerobic respiration is the respiration performed that is typically thought of in terms of breathing. Named aptly for its use of oxygen, aerobic respiration, more commonly known as cellular respiration, relies on the usage of oxygen to supply energy to the body's cells through glycolysis to the Krebs cycle to the Electron Transport Chain other various chemical pathways.

Interestingly, both the Krebs cycle and the Electron Transport Chain are explicitly found within the mitochondria nestled in the cells; the cells by themselves are anaerobic. Alternatively, when oxygen availability is significantly reduced in a localized environment, such as when a person is running, the body switches over to anaerobic respiration within the legs, which completes the process of glycolysis. Rather than continuing to the Krebs cycle, the byproducts undergo lactic acid fermentation, using pyruvate to produce lactate, ultimately allowing for NADH regeneration and the continuation of energy production through glycolysis. However, the introduction of microorganisms, some of which are beneficial and others pathogenic or hostile, into the human body or even individual cells could lead to some unpredictable consequences, most of which would be best to avoid. The next idea involves the use of viral vectors, which is currently being utilized quite heavily in genetics.

Viral vectors, as seen in epidermolysis bullosa where scientist took a skin sample from the patient and inserted genes, allowing the sample to grow in culture. When the sample had grown to a sufficient amount with the newly incorporated genes, they reattached the skin to the patient where the effects were resounding. The patient, "Bubble Boy", experienced decreased blistering as more connective tissue was created from the evolved skin graft to hold his skin in place. In theory, this could work, as a means of adapting the human body to live in space, but there is potential to insert DNA in wrong locations, many viruses associated with different cancers mention this, but the viral vectors would be directly evolved then inserted into cell culture population. Other things to consider are time, the process to wait for new cells to grow in culture, and practicality, is this the most efficient way to evolve the human body. This leads to probably the most feasible and probable options: the CRISPR Cas-9 system.

First discovered in bacteria as an anti-viral immune system of sorts, Drs. Charpentier and Doudna recently won a Nobel prize for their discovery of the CRISPR Cas-9 system. This intriguing finding is an innovative protein complex system that was first utilized for its natural abilities to capture pieces of an invading virus and then insert a small piece of the viral DNA into the bacteria DNA in order to have a memory of the virus and protect itself from the virus if it were to be infected again. Essentially, the CRISPR array in bacteria worked similarly to the lymphatic system within the human body. As with most neat innovations, the CRISPR system was later co-opted for its ability to make double-stranded DNA cuts in order to eliminate chosen genes, specifically those with non-desired expressed traits of genetically defined diseases. More recently, it has been further modified with different enzyme capabilities to be used in different ways, including as transcription activators and repressors.

There are two main components in the Cas-9 system, the Cas-9 protein itself, which provides the cutting mechanism, and the guide RNA that binds to the Cas-9 protein. The guide RNA acts as a locating beacon that searches and finds the specific palindromic sequence that has been specified to be cut. The entire system is highly specific, and one Cas-9 complex, the protein, and the guide RNA are custom made per individual gene. The unique mechanism and specificity of CRISPR Cas-9 provide a molecular toolbox for various alterations to the human genome to be made. For example, currently, researchers are exploring modifying the Cas-9 protein by inactivating it, meaning that it no longer has its cutting abilities, and fusing various enzymes such as a deaminase to the protein complex, which would allow for the induction of base substitutions or edits without breaking of the DNA double helictical complex. Fluorescent proteins can even be attached to the protein complex to allow some neat DNA imaging techniques! By altering the CRISPR toolbox and adding other proteins to CRISPR besides simply the cas9 complex the potential for increasing cardiovascular efficiency and inserting genes that aid in anticoagulation, with the goal of helping the body preserve water, in low water environments could be possible.

With the possibilities of CRISPR seemingly without end as more and more research and experimentation is done to free its hidden potential, an argument concerning the combining of CRISPR and directed evolution can be made. In a previous section where Dr. Arnold's technique of direct evolution was discussed, it was seen that Dr. Arnold and colleagues were inducing the direct evolution of the enzymes by purposely introducing mutations to the systems. What if, rather than humans, were bringing forth the random mutations, CRISPR did in an even more focused way. A hypothesis could be made that following a similar methodology of sequential mutagenesis, with the guide RNA acting as the site-directed phase and the inactivation of the Cas-9 protein along with a fused molecular component to enact the random mutagenesis phase, directed evolution would ensue without the need for active human involvement. Using CRISPR-cas9 or a variant of it along with the knowledge of the planet seeking to be inhabited, in this case, Mars, allows for a myriad of ways in which the body can be altered to become more suitable and adaptable to living in an unfamiliar environment.

What are some of the pitfalls of CRISPR, and can they be overcome in order to play their role in the directed evolutionary process of humans? Most of the overarching issues with CRISPR is the ethics. This is less and less science fiction and more science factual- people are doing this and it’s important to understand the potential, both good and bad. For example, Dr. Josiah Zayner has already injected himself with CRISPR, and publicly online, with the hope that he would grow bigger muscles through inhibiting myostatin gene. A more serious example is He Jiankui, the Chinese researcher who used CRISPR to knock out the CCR5 gene, linked to HIV, in embryo. The twins that were birthed as a result of this are currently living and growing as “normal” children, and the world waits to see the cascade of effects the result of one knockout would have on the rest of the human body.

While the issue of ethics will always remain in question, the use of directed evolution in tandem with CRISPR proves to be a sustainable route in bioforming the human body.

CHAPTER FOUR

Mars and Beyond

Thus far, we have discussed making extreme alterations to an extraterrestrial planet or extreme alterations to the human body itself. Moreover, while the science on each is potentially possible, both options detract from the aspects of what makes both living in space and being human alluring. If one were to terraform a planet, Mars, it would appear to be Earth's facsimile. If one were to begin making changes to the human form to fit the planet's natural environment, then the argument can be made that humans would no longer be humans but rather Martians. Ideally, a combination of both would be required, the best of both worlds, pun intended. If humans are to ever live on another planet aside from Earth, the best way and perhaps the most sustainable way would be to change the planetary environment and the forms of living organisms. This better maintains the goal of keeping the unique characteristics of Mars but with more habitable features and maintaining the essence of humanity, albeit with some slight superhero-esque abilities.

The chapter on terraforming explained how if one were to terraform Mars, it would require an intensive amount of mechanical engineering work to establish a self-sustaining ecosystem as seen so effortlessly on Earth. Overly manipulating the planet has the potential to lead to Earth 2.0 rather than truly colonizing Mars. From this research, it has been suggested to take focus away from Mars and potentially look at exoplanets that already exhibit Earth-like characteristics. Interestingly, these Earth-like characteristics are not the same as those mentioned in the previous chapter but instead require a more unorthodox approach should be taken. A change in perspective in the definition of habitability could be expanded to include planets that differ vastly from Earth, yet share traits to Earth's most extreme conditions, extreme conditions which we know aided in creating life, such as the hydrothermal vents. The goal of this is that, rather than looking for existing conditions of Earth currently, life should be spawned utilizing similar conditions found on early Earth.

Before this shift in thinking, the planets of interest have those in the Goldilocks' Zone. Named based on the children's bedtime story, the Goldilocks' zone is deemed a habitable range in which the temperatures, being demonstrated previously in the SpikerBox experiment as noteworthy, are just right enough to main a stable temperate environment as well as keep water in a liquid state at the planet's surface. Exoplanets such as Kepler-186f have been interested in discovering water and potentially finding life outside of Earth. While other planets have been found within this habitable region in space, Kepler-186f has been of particular interest since its size is most reminiscent of Earth's. In particular, Quintana et al. found that Kepler-186f is likely to have the necessary properties needed to hold reservoirs of liquid water due to a number of explored and calculated factors, including distance and orbital pattern around its star as well as the planet's composition based on photometry and thermal evolution modeling techniques (Quintana et al., 2014).

While research into the specific environment and atmosphere that Kepler-186f possess continues, it should be noted that just because an exoplanet resides in the Goldilocks' zone does not mean that its habitability can be assumed or is guaranteed, nor does an exoplanet residing outside of the Goldilocks' jurisdiction mean that it can be deemed unhabitable. With this realization, the reasoning towards what constitutes habitability, shifting towards the out-of-the-box thinking mentioned above, expands the finding of planets with conditions similar to early Earth rather than to the current form of Earth. With this at the forefront of the mind, it has led researchers to H2-rich atmospheric planets with massive oceans residing in their interiors, known as the Hycean Worlds. While not much is currently known about these exoplanetary worlds, the analysis done in Madhusudhan et al. leads to the potential that due their unique environment and biosignatures, Hycean Worlds could be a curious route in the journey to space habitation.

Finding a planet with more habitable features is a step in the right direction, however, changes must still be made to humans in order to achieve to goal of living in space without equipment. Things to consider would be if the planet had water but the water was frozen due to frigid temperatures. Anti-freezing techniques would need to be discovered, not just for the planet but for the living organisms as well. Typically, water freezes within the cell; it disrupts the structural organization of the cell as well as metabolic functions, which leads to cell death. However, observed freeze tolerance animals have restricted the freezing to only extracellular water by accelerated glucose release from hepatic glycogen stores. Glucose release raises the osmotic pressure of the extracellular space, which promotes cellular dehydration. Using the combined technique of CRISPR and directed evolution, perhaps genes can be added that allow for humans to restrict the freezing towards extracellular fluids as well, preserving the integrity of the human cells and life.

The idea of establishing an ecosystem outside of the realm of Earth is nuanced and complexed but finding commonality between planets, outside of what is seen on present Earth, but what has been seen on Earth as Earth has evolved could allow for breakthrough in the search for habitability in space. Once the right planet is found, humans and living organisms can then meet the needs of the planet half-way by making the necessary changes within in order to adapt to the planet's novel environment.

CHAPTER FIVE

If Space Colonization Were to Become the New Reality…

As exciting as everything that has been explored in this thesis would be, if it came into fruition within the next couple of years, it likely cannot and will not occur. It will probably not occur within this lifetime for various practical and other ethical reasons, primarily because there are so many questions yet unanswered. Perhaps humans will never be ready because there is always a gray area. Some issues leave people feeling perplexed, where they can see both sides of an argument and no definite answer can be made. This is currently one of those situations. Should humans have the authority or power to colonize planets outside of Earth? If people begin enhancing themselves and do not go and live in space where the enhancements were meant to be essential, is that fair to those who are not "super"? This thesis was not written to be a resounding proposition for space colonization but merely a scientific exploration of the possibilities that lay before the human race. It is only fair that the discussion concludes with possible rebuttals to potential inquiries many will undoubtedly raise when the gray area finally decides to be crossed.

Perhaps the most salient question is regarding those human modifications and the potential consequences that arise when there becomes a distinction between humans and *humans*. To clarify, the modifications made to the human body would focus on the single intent of making life on Mars or any other planet of choice a natural and sustainable possibility. However, good intentions do not equate to good consequences, and because of this, the issue of eugenics comes into play.

Eugenics is classically the study of how to arrange human reproduction so that heritable characteristics are the most desirable, with a purposeful selection of genes that are deemed desirable. At first glance, this does not appear to be the worst thing. One may think that selecting good; advantageous traits would be what was wanted. In a way, this is true, but by selecting only for the traits that are deemed "good," a significant consequence of this selection is a decrease in the biodiversity pool of genes, which would then be specifically within the human population—is for this reason, why inbreeding, besides being morally taboo, is frowned upon.

In the wrong minds and hands, an idea demonstrated with in the He Jiankui CRISPR case, the proposed mechanism for bioforming the human body could be potentially used to carry out some twisted eugenics plan. While it may seem that directly evolving humans for traits that make them humanly adaptable to an extraterrestrial atmosphere is a form of eugenics, it could be argued that it is, in fact, the opposite. By using a directly evolved CRISPR system, one is not seeking to decrease the biodiversity of humans and make a more "perfect" human, who does not genuinely exist. Instead, one is increasing the gene pool, allowing for the option for people to choose to have these characteristics so that their bodies can be adaptable to a space environment.

What if people are on board with the idea of making enhancements to the human body, and the issue is not of ethics, what would be the problem then? From a neuroscientific background, what could be the possible physiological stressors that would be placed on the body because of these changes. It is unclear what those types of changes to the body might do to other organ systems that are not being directly evolved and the change in dynamic and interactions between the evolved systems versus the non-evolved systems. Specifically, looking at the central nervous system, the control system of the body, what type of mental stressors would that place on the body itself if these changes were to be made. As Newton's third law of motion states, for every action there is an equal and opposite reaction, repercussions from these changes are to be expected, and because of the novelty of the idea there is no clear theory or expectation in determining what those effects might look like when there is nothing from the past or currently reality that it can be compared to.

Moving away from the ethics of evolving humans and the potential consequences on the human body if those changes were to be made, is it ethical to introduce alien life to a new planet with an already existing ecosystem? Thinking about how humans have propelled the onset of global warming, what potential ecological disasters could ensue if life was introduced on a planet not intentionally meant for life? What would the implications be for introducing alien life to a planet with an already existing ecosystem? The implications of this are not truly known as a venture like this has not be embarked on before, but examples form what has occurred to earth such as the dissolution of limestone, can be used to inform the hypotheticals, which would then guide decisions on whether it is deemed ethical.

Lastly, this thesis does not take in to account the necessary technological advances that would be needed to reach the planets in a short enough time, nor does it focus on the anatomical and physiological changes that would be necessary to withstand that amount of space travel. These are all necessary things that would need to be considered, were humans to inhabit space. What would that look like, would humans need to enter a stasis mode similar to tardigrades or be placed in cryogenic hibernation capsules as seen on the spaceships in *Star Wars*. While it would have been neat to explore it further, the goal of thesis was act as a launching pad and begin having others build and think upon the very real potential of living in space, a direction in which the current world is already thinking of. Emphasis is on new, original ideas on how it might be possible for an ecosystem to reside outside of Earth. The hope is that by reading this thesis, the reader realizes that for people to believe in directed evolution and its properties, beyond the realm of macromolecules, we need to stop thinking that certain things are solely unique to planet Earth and humans in general. In *The Zoologist's Guide to the Galaxy*, author, Arik Kershenbaum noted that it is very possible that on other planets with their own already potentially existing ecosystems, natural selection is the driving force. Natural selection may very well be the driving force of evolution in the universe, but it may occur differently that what is known on Earth. The idea is that natural selection is not unique to Earth but occurs everywhere, employing different techniques due to different environments. As Kershenbaum poignantly said "Nature is not unique to the visible world" and because of this hopefully, the realm of what is possible can be unearthed further (Kershenbaum, *The Zoologist's Guide to the Galaxy: What Animals on Earth Reveal about Aliens--and Ourselves*).

BIBLIOGRAPHY

Arnold, Frances H. "Innovation by Evolutio: Bringing New Chemistry to Life (Nobel Lecture)." *Angewandte Chemie - International Edition* 58 (2019): 14420–26. https://doi.org/10.1002/anie.201907729.

Berman, Chet M., Louis J. Papa III, Samuel J. Hendel, Christopher L. Moore, Patreece H. Suen, Alexander F. Weickhardt, Ngoc-Duc Doan, et al. "An Adaptable Platform for Directed Evolution in Human Cells." *Journal of American Chemical Society* 140 (2018): 18093–103. https://doi.org/10.1021/jacs.8b10937.

Chen, Kequin, and Frances H. Arnold. "Tuning the Activity of an Enzyme for Unusual Environments: Sequential Random Mutagenesis of Subtilisin E Catalysis in Dimethylformamide." *Proc. Natl. Acad. Sci. USA* 90 (June 1993): 6618–5622.

"Curiosity Rover Serves Scientists a New Mystery: Oxygen | NASA." Accessed April 22, 2022. https://www.nasa.gov/feature/goddard/2019/with-mars-methane-mystery-unsolved-curiosity-serves-scientists-a-new-one-oxygen.

Davis, J. Mark, Zuowie Zhao, Howard S. Stock, Kristen A. Mehl, James Buggy, and Gregory A. Hand. "Central Nervous System Effects of Caffeine and Adenosine on Fatigue." *AJP-Regul Integr Comp Physiol* 284 (October 24, 2002): 399–404.

Climate Change: Vital Signs of the Planet. "Earth's Atmosphere: A Multi-Layered Cake." Accessed April 22, 2022. https://climate.nasa.gov/news/2919/earths-atmosphere-a-multi-layered-cake.

"Earth's Magnetosphere | NASA." Accessed April 22, 2022. https://www.nasa.gov/magnetosphere/.

Eigen, Manfred, and William Gardiner. "Evolutionary Molecular Engineering Based on RNA Replication." *Pure and Appl. Chem.* 56, no. 8 (1984): 967–78.

Galland, Leo. "The Gut Microbiome and the Brain." *Journal of Medicinal Food* 17, no. 12 (2014): 1261–72. https://doi.org/10.1089/jmf.2014.7000.

Gautam, Pallavi. "Divergent Evolution." In *Encyclopedia of Animal Cognition and Behavior*, edited by Jennifer Vonk and Todd Shackelford, 1–8. Cham: Springer International Publishing, 2020. https://doi.org/10.1007/978-3-319-47829-6_501-1.

Graham, James M. "The Biological Terraforming of Mars: Planetary Ecosynthesis as Ecological Succession a Global Scale." *Astrobiology* 4, no. 2 (2004): 168–95.

Gregory, T Ryan. "Understanding Natural Selection: Essential Concepts and Common Misconceptions," 2009, 20.

Juneja, V, L Huang, and H Thippareddi. "Predictive Model for Growth of Clostridium Perfringens in Cooked Cured Pork☆." *International Journal of Food Microbiology* 110, no. 1 (July 1, 2006): 85–92. https://doi.org/10.1016/j.ijfoodmicro.2006.01.038.

Kershenbaum, Arik. The Zoologist's Guide to the Galaxy: What Animals on Earth Reveal about Aliens--and Ourselves, n.d.

Laboratory, Sizing Up Humanity's Impacts on Earth's Changing Atmosphere: A. Five-Part Series By Alan Buis NASA's Jet Propulsion. "The Atmosphere: Earth's Security Blanket." Climate Change: Vital Signs of the Planet. Accessed April 22, 2022. https://climate.nasa.gov/news/2914/the-atmosphere-earths-security-blanket.

Linse, Sara Snorgerup. "Directed Evolution of Enzymes and Binding Proteins." *Kungl. Vetenskaps-Akademien (The Royal Swedish Academy of Sciences)*, 2018, 1–24.

Lopez-Garcia, Purificacio, and David Moreira. "The Syntrophy Hypothesis for the Origin of Eukaryotes Revisited." *Nature Microbiology* 5 (May 2020): 655–67. https://doi.org/10.1038/s41564-020-0710-4.

Madhusudhan, Nikku, Anjali A. A. Piette, and Savvas Constantinou. "Habitability and Biosignatures of Hycean Worlds." *The Astrophysical Journal* 918, no. 1 (September 1, 2021): 1–25. https://doi.org/10.3847/1538-4357/abfd9c.

Moreira, David, and Purificacion Lopez-Garcia. "Symbiosis Between Methanogenic Archea and Delta-Proteobacteria as the Origin of Eukaryotes: The Syntrophic Hypothesis." *Journal of Molecular Evolution* 47 (1998): 517–30.

Oberheim, Nancy Ann, Takahiro Takano, Xiaoning Han, Wei He, Jane H. C. Lin, Fushun Wang, Qiwu Xu, et al. "Uniquely Hominid Features of Adult Astrocytes." *The Journal of Neuroscience* 29, no. 10 (March 11, 2009): 3276–87. https://doi.org/10.1523/JNEUROSCI.4707-08.2009.

Quintana, Elisa V., Thomas Barclay, Sean N. Raymond, Jason F. Rowe, Emeline Bolmont, Douglas A. Caldwell, Steve B. Howell, et al. "An Earth-Sized Planet in the Habitable Zone of a Cool Star." *Science* 344 (April 18, 2014): 277–80.

Sleator, Roy D., and Niall Smith. "Terraforming: Synthetic Biology's Final Frontier." *Archives of Microbiology* 201 (2019): 855–62. https://doi.org/10.1007/s00203-019-01651-x.

Spina, Lorenzo. "Chemical Evidence for Planetary Ingestion in a Quarter of Sun-like Stars." *Nature Astronomy* 5 (2021): 7.

Stefanutti, Eleonora. “Structural and Dynamical Studies on Confined Water.” Doctoral Thesis, Universite Pierre et Marie Curie, 2019.

Steigerwald, Bill. “MAVEN Maps Electric Currents around Mars.” Text. NASA, May 22, 2020. http://www.nasa.gov/press-release/goddard/2020/mars-electric-currents.

Stemmer, Willem P. C. “Rapid Evolution of a Protein in Vitro by DNA Shuffling.” *Nature* 370, no. 6488 (August 1994): 389–91. https://doi.org/10.1038/370389a0.

Tawfik, Dan S., and Andrew D. Griffiths. “Man-Made Cell-like Compartments for Molecular Evolution.” *Nature Biotechnology* 16 (July 1998): 652–56.

Tycko, Josh, Gaelen T. Hess, Edwin E. Jeng, Michael Dubreuil, and Michael C. Bassik. “The Expanding CRISPR Toolbox.” *Nature Methods*, n.d.

Waltham, David. “Is Earth Special?” *Earth-Science Reviews* 192 (2019): 445–70.

“What Is a Magnetic Field? - Universe Today.” Accessed April 22, 2022. https://www.universetoday.com/76515/magnetic-field/.