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Thermodynamic perspectives on genetic instructions, the laws of biology and diseased states

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ABSTRACT

This article examines in a broad perspective entropy and some examples of its relationship to evolution, genetic instructions and how we view diseases. Living organisms are programmed by functional genetic instructions (FGI), through cellular communication pathways, to grow and reproduce by maintaining a variety of hemistable, ordered structures (low entropy). Living organisms are far from equilibrium with their surrounding environmental systems, which tends towards increasing disorder (increasing entropy). Organisms free themselves from high entropy (high disorder) to maintain their cellular structures for a period of time sufficient to allow reproduction and the resultant offspring to reach reproductive ages. This time interval varies for different species. Bacteria, for example need no sexual parents; dividing cells are nearly identical to the previous generation of cells, and can begin a new cell cycle without delay under appropriate conditions. By contrast, human infants require years of care before they can reproduce. Living organisms maintain order in spite of their changing surrounding environment that decreases order according to the second law of thermodynamics. These events actually work together since living organisms create ordered biological structures by increasing local entropy. From a disease perspective, viruses and other disease agents interrupt the normal functioning of cells. The pressure for survival may result in mechanisms that allow organisms to resist attacks by viruses, other pathogens, destructive chemicals and physical agents such as radiation. However, when the attack is successful, the organism can be damaged until the cell, tissue, organ or entire organism is no longer functional and entropy increases.

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1. Introduction

Atoms are ancient relics of the hypothesized Big Bang (Matsuno, [1]) and can be used to construct life forms under the control of FGIs (functional genetic instructions). Living organisms are programmed by FGIs, which flow through a biochemical communication pathway involving DNA–> RNA–> proteins, to instruct cells how to assemble into living organisms. They are programmed to grow and reproduce by maintaining a variety of hemistable, ordered structures (low entropy state) (Schrodinger, [2]). They are far from equilibrium with their surrounding environment, which tends towards increasing disorder (Dolev and Elitzur, [3]). This is achieved by absorption of energy, from our thermonuclear sun, which provides the energy for the conversion of inanimate material into living organisms. This occurs on our planet with conditions

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commensurate with the maintenance of the life forms that comprise our singular biosphere system (Dolev and Elitzur, [3]; Gatenby and Frieden, [4]).

Researchers have devoted time and effort to defining and understanding the characteristics of life, from the atomic to the biospherical levels of organization (Penzlin, [5]; Schrodinger [2]) and in more recent years the possibility of synthetic single-celled life. Biology can therefore be viewed as the study of life (and death) at all levels of biological organization. The study of dead organisms and the process of dying reveal much about living organisms. We can apply what we learn from studying dead organisms, cells and tissues to experiments in microbiology, pathology, forensics and the treatment of diseases. Science relies on the fundamental laws of thermodynamics in addition to the knowledge that: (1) the cell is the basic unit of life; (2) life arises only from life; (3) a cell is the only living structure that can grow and divide (Trevors, [6]), and (4) functional genetic instructions flow along a cellular communication pathway to provide the instructions for the challenges from entropy, with reproduction as the normal outcome. Although natural selection prevents many individual organisms from reproducing, others must succeed if a species is to survive, even though all individuals within a species die, generally just not at the same time. Species extinction statistics attest that natural selection has removed an alarming number of species from our biosphere. However, with a population of about 6.8 billion, humans trump natural selection as the primary culprit in species extinction. The human population is increasing by about 75–80 million people annually, assaulting our singular, shared biosphere, eliminating other species and warming the planet.

Fortunately humans also have the gift of imagination that can be used in creative and unfortunately, destructive ways. While some humans limit their imagination largely to the invention of supernatural explanations for what they do not understand (i.e., gods, spirits, supernatural, unexplained phenomena), others eagerly explore the arts, business, science, medicine, engineering and other disciplines, regardless of their perspective on the supernatural. But no matter how people imagine our world, reality is governed by natural laws with thermodynamics the most fundamental of all laws. Moreover, it is known that functional genetic instructions (FGI) in genomes are the instructions for each respective life form. Therefore, one rational approach to understanding life involves studying the evolving interconnectivity of FGI in living organisms. Evolution and disease (a form of natural selection and a battle with entropy) are viewed as integral parts of biology. By knowing that organisms can both use and store energy, and both use and preserve FGI in their genome with relative constancy, yet some change over time, we begin to understand the hallmarks of life itself with growth and reproduction the desired or normal ultimate outcomes.

2. The three laws of biology

The First Law of Biology: All living organisms obey natural laws. This law is fundamental because the laws of the inanimate universe determine the course of the universe. All organisms obey these laws. The laws of thermodynamics govern energy transformations and mass distributions. Cells that comprise living organisms are semi-permeable, open systems (von Bertalanffy, [7]) that allow both mass and energy to cross their membranes. Cells exist in open systems so as to allow acquisition of elements, nutrients, and also novel genetic traits while extruding end products of metabolism and toxic substances. Genetic variation, which results in part from gene transfer in prokaryotes and sexual reproduction in higher organisms, allows increased phenotypic variability in a population as well as accelerated rates of evolutionary divergence.

A corollary of the First Law is that life requires the temporary creation of order or lower entropy (less randomness) in apparent contradiction to the second law of thermodynamics. However, considering a living system, including the materials and energy sources provided by the environment for the maintenance of life, living organisms affect the system strictly according to this law, by increasing randomness or chaos (entropy). Resource utilization by living organisms thus increases the entropy of the world. A second corollary of the First Law is that an organism at biochemical equilibrium is dead. When living organisms reach equilibrium with their surrounding environment, they no longer exhibit the characteristics of life. Life depends on interconnected biochemical pathways to allow for growth, macromolecular synthesis, and reproduction. Thus, all life forms are far from equilibrium with their surrounding environmental systems.

The Second Law of Biology: All living organisms consist of membrane-encased cells. Enveloping membranes allow physical separation between the living and the non-living worlds. Viruses, plasmids, transposons, prions are not alive, even though they may be reproduced independently of organismal reproduction. However, they cannot "self" reproduce. They are dependent on a living cell for this purpose. By definition, they therefore, are not alive. A corollary of the Second Law is that the cell is the only structure that can grow and divide independently of another life form. Cell division depends on the cellular envelope structure and a large number of macromolecular types that interact in a functionally cooperative way and were encoded by the correct genetic instructions. Therefore, a second corollary of the Second Law is that all life is programmed by organic, genetic instructions. Genetic programming is required for cell division, morphogenesis and differentiation. From single-celled prokaryotic organisms to normal or cancerous tissues in multicellular animals and plants, genetic instructions are required.

The Third Law of Biology: All living organisms arose in an evolutionary process. This law correctly predicts the relatedness of all living organisms on Earth. It explains their programmed similarities and differences. Organisms can live, reproduce and die. If they die without reproducing, their genes are usually removed from the gene pool, although exceptions exist such as in bacterial DNA genetic transformation. At the molecular level, genes and their encoding proteins can evolve "selfishly," and these can combine with other selfish genes to form selfish operons,

genetic units and functional parasitic elements such as viruses. Two corollaries of the Third Law are that (1) all living organisms contain homologous macromolecules (e.g., DNA, RNA, and proteins) that are derived from a common ancestor, and (2) the genetic code is universal. These two observations provide compelling evidence for the Third Law of Biology. Because of his accurate enunciation of the Third Law, Charles Darwin is considered to be the greatest biologist of all time.

Although progress has been made in understanding past and present life forms, the origin of life, the location(s) where life arose, and the origin of genetic instructions embedded in DNA and RNA remain a mystery (Trevors, [8,9]). What we are certain of: construction the first cells, and all subsequent species, were governed by the most fundamental of all laws – the laws of thermodynamics. In addition, life operates within the growth cycle: cell division is accompanied by reproduction, and death is the consequence, whether programmed or accidental.

In order to understand how life deals with higher entropy environmental systems, we must examine how the laws of thermodynamics govern growth and reproduction *via* programs of gene expression. FGI guides cells through the cell cycle in processes requiring specific instructions. When mistakes (genetic errors) are made, such as a lethal mutation, entropy wins and the organism dies, thus ensuring that only the fittest FGI remain. Without knowing ahead of time what the future environment will be, the key to species survival is the ability of some individual organisms to adapt to changing environmental conditions and reproduce. At the core of thermodynamically possible organisms, FGI would have been required for the first minimal cells. Thus an organism can either live and die without reproducing or live to reproduce. Natural selection offers only these two choices when life is waging the challenge to survive against entropy.

3. Functional genetic instructions (FGI)

Life as we know it is not possible without FGI (Abel and Trevors, [10–12]). Living organisms are alive because FGI maintains stable ordered (low entropy) states. A transmembrane potential permits cells to perform specific metabolic functions by importing nutrients and exporting wastes. Since cells are semi-permeable but open systems, they must accurately identify and obtain diverse molecules and atoms to synthesize without excessive genetic mistakes. In fact, regulated DNA replication, transcription and translation can be viewed as a biochemical communications pathway that allows the FGI to assemble an organism with reproduction as the ultimate objective. Using bacteria, an example of thermodynamic efficiency can be understood. When the thermodynamic efficiency of bacterial growth for *Pseudomonas oxalaticus* under C-limited chemostat conditions was estimated, the conclusion was that some bacteria have been optimized for a thermodynamic efficiency of 23 to 24%. This estimate was based on the C-mol dry weight produced per C-mol carbon substrate consumed. For citrate-limited growth for *Bacillus licheniformis,* the value was estimated at 28% (Rutgers, et al., [13]). The thermodynamic efficiency depends on the degree of reduction of the carbon substrate. For example, autotrophs and heterotrophs would also exhibit different optimal values. However, predictions based on thermodynamic methods are not without their limitations. For example, one or more substrates can be metabolized simultaneously, not all catabolic reactions are known in all organisms, and environmental growth conditions fluctuate over time scales and locations in nature. Therefore, similar thermodynamic efficiency values for a multitude of diverse organisms have not been estimated.

It is important to understand the semantic separation of the functional genetic message from the biochemical molecules that transmit the message along a pathway. The genetic message is embedded in the linear, biochemical, structure of DNA and RNA. The information within the genetic code is independent of the elements that comprise the DNA molecule. It is the sequence of bases (codons), not the nature of bases that determines the code. Thus the message is independent of the medium just as the message, "The Earth is not flat," does not change whether it is written in crayon, ink, chalk or pencil. It can even be translated into the dots and dashes of Morse code, but the information transmitted remains the same. FGI is not free – it comes at a cost to living organisms (Lineweaver and Egan, [14]). That cost is provided by intermediary metabolism, which provides energy and molecular building blocks. Even the formation of a bacterial spore to protect the FGI comes at a cost required to preserve the genetic message (*via* spore formation) under adverse environmental conditions.

Synthesizing a cell from the surrounding environment, whether it is a bacterium in soil or a cell in a healing wound, requires an input of energy to decrease entropy. When a cell dies and order gives way to disorder, the energy and matter are returned to the system (Pollack, [15]). Since the laws of thermodynamics existed prior to life on Earth, the first cells and all subsequent cells had to contend with entropy to evolve, as structural order is the hallmark of life and entropy is the forward direction of time.

The transition from an anaerobic Earth to an oxygen environment was monumental. As more energy became available from aerobic metabolism, aerobic prokaryotic organisms may have grown and reproduced more efficiently. Mitochondria may have provided an evolutionary leap in eukaryotes (Lane and Martin, [16]) toward more efficient production and storage of energy, which sped up the evolution of specialized cells, tissues and organs, leading to the diversification of species. Evolving organisms were winning the challenge against entropy! The splitting of ATP to yield about 7 kCal/mol was just what life needed to evolve. The evolutionary process, though amazing, is not perfect and not without lethal mistakes that yielded to fierce natural selection.

4. Diseases and genetic instructions

All known life forms depend on having the correct FGIs maintained in their cells. FGIs are algorithms that produce the linear organic, genetic sequence that in turn produces

the correct molecular conformations and activities in living organisms. Although FGI can resist some attacks by viruses, chemicals and physical agents such as UV light, when the attack is successful, the genome can be damaged beyond repair. For example, tumor cells may be preserved and proliferate when normal FGI is disrupted in some cells, tissues or organs. The tumor can be removed, irradiated and attacked with chemicals in an attempt to kill it and restore normal FGI. However, if the FGI is permanently damaged, it is difficult to halt progression of the disease and death of the organism.

It is also possible for a virus to genetically take over a cell and destroy its normal function by damaging the genetic instructions. The cells will try to maintain normal entropy, but if the attack succeeds, metabolic order will become disordered and loss of normal metabolic function will occur. No matter how it occurs, from an evolutionary perspective, destroying FGI in an aging organism is of less significance compared to maintenance of the FGI in an organism that can still reproduce and perpetuate the species.

The transformation of energy and matter in living organisms occurs when FGIs are passed on to the offspring of the species. Put simply, at the molecular level, the biosphere among other things is an immense biochemical gene factory containing enormous amounts of genetic instructions operating under the laws of thermodynamics. For example, Markos [17] proposed that Gaia is a planetary information network with microorganisms having a major role in the Earth as an evolving homeostatic system (Gaia theory, Lovelock, [18]).

We are still in the early stages of understanding the relationship between FGI and thermodynamics (Dolev and Elitzur, [3]). The first law of thermodynamics states that energy is always conserved. The second law states that within a closed system (where only energy can enter or leave the system, while matter cannot), the amount of randomness or disorder, known as entropy, must increase. Are there any possible exceptions? Wang et al. [19] reported experimental data (supporting the fluctuation theorem that dissipative flux flows directionally reverse to that in the second Law of Thermodynamics) that demonstrated the Second Law of Thermodynamics was violated in a small system and over short periods of time. Entropy was negative for tenths of seconds when mm scale latex beads gained energy from the random motion of water molecules. When the time scale was greater than 2 seconds, the overall entropy change was again positive and in agreement with the second law. Small scale systems not behaving like larger systems has interesting implications for scaled-down nanotechnology devices and a better understanding of the origin of life and living cells such as bacteria in the micron size scale. Quite simply, we need more research on such important observations.

On the other hand, in an open system, both matter and energy can leave and enter the system. Cells with their semi-permeable membranes are open systems (Trevors and Pollack, [20]), otherwise metabolism would not be possible, and hence life would not be possible. Some cells, such as bacteria, also can acquire novel genetic information via transformation, conjugation and transduction. If this was not the case, the evolution of bacteria would be much slower. Entropy is the opposite of the more ordered living state. It is the uphill challenge that living organisms have fought for about 4 billion years to bring us to the current state of our biosphere (Trevors, [21]). Of the estimated 13 billion years (maybe older) of the universe's existence, it took perhaps 8 billion years or more to create the universal toolbox of elements and molecules necessary for the origin and subsequent evolution of life on the Earth.

Life at the molecular level is instructed to grow and reproduce. In organisms, FGI contains the instructions for gene expression, thus maintaining the low entropy, homeostatic state necessary for organisms to survive and reproduce. For example, the singular bacterial cell, with several thousand genes, actually exhibits higher entropy than a human with diverse systems, tissues, organs and a brain. This is possible because of the correct FGI and the amount of energy (metabolism) invested is less than the return. The entropy of the organism decreases to allow reproduction, while the entropy of the surrounding environment increases.

Each species has the correct amount of FGI to wage the challenge against entropy. If the organism is damaged or injured or the FGI are damaged by a virus, bacteriophage, mutagen, radiation, chemicals, heat or cold, the challenge can be lost. If the damage passes a certain threshold, and repair is not possible, the outcome is usually death of the organism. Natural selection is brutal and painful. As humans, we intervene in natural selection to preserve the health/life of humans, animals, plants and other organisms. Still, as many species become extinct, our biosphere's biodiversity suffers. The strict relationship between the most fundamental of all laws, thermodynamics, and FGI in organisms, emerges as central to our knowledge and understanding of organisms, evolution and diseases.

What if each species could be assigned a mathematical value (or barcode) that describes the relationship between the challenge with entropy and the genome size or FGI contained in the genome? This value could be a possible estimate of the entropy or complexity for that species. Such a value could be used in research and ultimately for humanity, possibly revealing evolutionary relationships between species or providing information on the normal versus diseased, injured or altered state, although, at this point, this is only speculation. However, problems exist as individuals in each species have slightly different genomes. The mass, volume and body temperature, heat output, water content and respiration combined with genome size, could be used as starting points to determine if useful relationships exist. On a smaller scale, the functional sequence complexity of some proteins has already been estimated (Durston, et al., [22]). Entropy has also been proposed as the ratio between signal perturbation and the total signal in medicine applied to mammogram analysis (Vitulano and Casanova, [23]). For example, a malignant mass with a diseased structure is defined by a high entropy value.

It is not a new concept to view cancer through the lens of evolution (Merlo, et al., [24]). Looking at the disease as an attack on FGI, entropy or disorder is increased when

cells and tissues cannot function correctly and homeostasis is defeated. Cancer treatments force cancerous cells in a thermodynamic direction, where growth and division are not possible, halting further development of the damaged cells. Not only is cancer an evolutionary and ecological process (Merlo, et al., [24]), it is also a thermodynamic entropy and homeostasis process. Evolution favors organisms with low entropy over higher entropy (Sabater, [25]), resulting in the more optimal state of homeostasis. Diseases, such as cancer, interfere with this process and increase entropy as the normal functions of the cells are disrupted. Uncontrolled growth is incompatible with homeostasis metabolism as increased genetic variability and mistakes move the organism further away from normal thermodynamics. Entropy is a time signature for the forward march in time (Stenholm, [26]); therefore entropy can only increase as time progresses, and as such, time and entropy are irreversible. Eventually entropy trumps life, even synthetic systems. Placing values on entropy is also problematic because there are no absolutes (Stenholm, 2008). Moreover, the concept of entropy tells us that the universe is moving from order to disorder. Before the Big Bang, order would have been at its maximum.

Conflict of interest statement

The authors have no conflict of interest.

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