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Fundamental Taboos of Biology

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Abstract—This paper formulates some taboos relating to living systems and cognition of these systems: in nature, there exist no two identical living complex multicellular organisms; there is no way to create an exact copy of a multicellular organism; there is no way to obtain two identical clones of a unicellular organism if they contain a sufficiently large number of cells; based on comparing present-day organisms, it is impossible to restore the structure of the first living cell and the processes that have led to its emergence; it is impossible to create a living cell from its separate simple constituents; the mechanisms determining cell vitality are essentially incognizable.

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Among common questions on the way to the biological Holy Grail, i.e. to the understanding "what life is", the question "are there any fundamental laws in biology?" is the most important.

The formulation of such laws seems to be inevitable at least for a primary approach to the cognition of the essence of life. "Physicists come from a tradition of looking for all-encompassing laws, but is this the best approach to use when probing complex biological systems?" – Evelyn Fox Keller asks in an interesting essay [1] recently published in *Nature*. And she proceeds: "Biologists often pay little attention to debates in the philosophy of science. But one question that has concerned philosophers is rapidly coming to have direct relevance to researchers in the life sciences: are there laws of biology? That is, does biology have laws of its own that are universally applicable? Or are the physical sciences the exclusive domain of such laws?" Below, I will briefly present Keller's further reasoning.

To collate lots of facts accumulated due to successes in genomics and to understand how proteins, genes, and other components of the living cell interact with each other in the context of complex networks, modeling, and other quantitative tools, well established in physical sciences, are beginning to be widely used. This raises natural questions as to how much specificity needs to be included in these models for each organism (species), where simplifying assumptions are appropriate, and to what extent the search for general laws of biology is generally useful. In view of the increasing wave of information, these questions have now acquired practical importance and even urgency.

In the past, biologists have been little concerned about whether their findings might achieve the status of a law. And even when findings seemed to be so general as to warrant thinking of them as a law, the discovery of limits and exceptions to their generality has not been seen as a problem. For example, exceptions to Mendel's laws or the "law" of natural selection were no cause for alarm and did not stimulate biologists to search for more general, exception-free laws. Exceptions were simply reminders of how complex biology is in reality. And this is different from physics, where the search for universal laws was always a high priority. Moreover, physics gave birth to the belief that the universal laws are *sine qua non* (essential condition) of a proper science. Physical and biological approaches have coexisted separately for almost a century, but today, when physicists, mathematicians, computer scientists, and engineers come to work in biology, and when many new institutes, departments, and centers spring up under the flag of system biology, the problem of the convergence of different attitudes towards the general and the particular comes to the fore.

So, how appropriate is it to look for all-encompassing laws to describe the properties of biological systems? By its very nature, life is both contingent and particular, and each organism is the product of millions of years of tinkering, of building on what had accumulated over the course of a particular evolutionary trajectory specific for each species. Of course, the laws of physics and chemistry

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are crucial. But, beyond these laws, biological generalizations (with the possible exception of natural selection) may need to be provisional because of evolution and the historical contingencies, on which both the emergence of life and its elaboration depended.

"Perhaps", Keller adds in conclusion, "it is time to face the issues head on, and ask just when it is useful to simplify, to generalize, to search for unifying principles, and when it is not. There is also a question of appropriate analytical tools. ... These are hard questions, but they may be crucial to the forging of productive research strategies in systems biology. Even though we cannot expect to find any laws governing the search for generalities in biology, some rough, pragmatic guidelines could be very useful indeed."

Here is one more idea suggested by another author in the same vein: "Recently, ideas about complexity, self-organization, and emergence — when the whole is greater than the sum of its parts — have come into fashion. ... But such explanations offer only smoke and mirrors, functioning merely to provide names for what we cannot explain. ... Perhaps there can be a general theory of complex systems, but it is clear we do not have one yet" [2].

As to if such a theory can be developed in principle, there are also different points of view. For example, Lamarck [3] wrote (translation from Russian): "I believe it is much easier to describe the movement of stars observed in space, to determine the distance, size, mass and movement of planets in our solar system than to solve the problem of the *source of life* in living bodies and hence of the origin and creation of different existing living bodies. But however difficult the great task of our searches is, its difficulties are not insurmountable as in this entire in problem one deals only with purely *physical* phenomena". Lamarck thus stated the possibility of knowing the causes (i.e. the laws) of life.

On the other hand, such a prominent physicist as Niels Bohr suggested incognizability of life as follows: "Thus we should doubtless kill an animal if we tried to carry the investigation of its organs so far that we could tell the part played by the single atoms in vital functions. ... Minimal freedom, which we must allow the organism, will be just large enough to permit it, so to say, to hide its ultimate secrets from us. On this view, the very existence of life must in biology be considered as an elementary fact, just as the existence of the quantum of action has to be taken as a basic fact that cannot be derived from ordinary mechanical physics" [4]. Indeed, even without killing, just by introducing an instrument into a living system we so much affect its properties that study not the system itself but a result of its interaction with the instrument in the region of this interaction. This is the uncertainty principle in biology, similar to that in physics. Since it is principally impossible to prevent the interaction of the electron with the instrument used to study its properties, the electron's position and momentum cannot be simultaneously determined to arbitrary precision. However, the uncertainty principle is still one of the fundamental physical laws.

Iosif Shklovsky, a remarkable soviet astrophysicist, expressed the following point of view (surely, he did not expect to be quoted): "Science is a sum of taboos. It is impossible to create a perpetuum mobile. One cannot transmit a signal with a speed higher than the speed of light in vacuum, and there is no way to simultaneously measure the speed and coordinates of the electron" [5]. This is a very elegant definition, though certainly not sufficient. Nevertheless, this definition prompts a possible way to define some fundamental laws not only in physics — I mean fundamental prohibitive laws. And then one can ask if there are any taboos in biology.

To my mind, one of such fundamental prohibitions is obvious. ***In nature, there exist no two identical living complex multicellular organisms. There is no way to create an exact copy of a multicellular organism. There is no way to obtain two identical clones of a unicellular organism if they contain a sufficiently large number of cells.*** This law follows from an extreme lability of the genetic material when even genomes of individual cells of one and the same organism acquire genetic and epigenetic differences from each other throughout the development from the fertilized egg. These differences evolve stochastically at many levels and can be grouped into two main categories.

Level of hereditary changes including assortment, mutation, and rearrangement of chromosomes in the process of gametogenesis. I will start this part with a citation from a book by E. Wilson "On Human Nature" that reads as follows: "Since each individual produced by the sexual process contains a unique set of genes, very exceptional combinations of genes are unlikely to appear twice even within the same family. So if genius is to any extent hereditary, it winks on and off through the gene pool in a way that would be difficult to measure or predict. Like Sisyphus rolling his boulder up to the top of the hill only to have it tumble down again, the human gene pool creates hereditary genius in many ways in many places only to have it come apart in the next generation" [6].

Here Wilson talks about classical genetic stochastics at the level of gametogenesis, i.e. independent assortment into gametes (germ cells) of the chromosomes obtained by the individual from father (23 different chromosomes) and mother (23 chromosomes, very similar but not identical to those of father). Each gamete bears 23 chromosomes, each one from either the paternal or maternal set. Let us assume that one gamete obtains chromosome 1 from the paternal set, chromosomes 2 and 3 from the maternal set, etc., and another gamete has chromosomes 1 and 2 from the maternal set, chromosome 3 from the paternal set, etc. This is Mendel's law of independent assortment. As a result, each gamete has its particular combination of paternal and maternal chromosomes. There can be 8,388,608 different combinations of 23

chromosomes (Wikipedia, Mendelian inheritance). The gametes will be then fertilized and will give rise to descendants of the individual in which they were formed. Descendant A will have one of many possible combinations of chromosomes, while descendant B of the same parent will have another one. Although the process is stochastic, the result can be easily predicted: descendants A and B will bear exact resemblance neither to each other nor to their parents. An exception is monozygotic twins. This is the classical Mendel's stochastics, i.e. stochastics at the level of chromosomes that are transferred to the progeny as whole units.

However, in reality chromosomes behave differently. They always mutate, rearrange, and recombine during the process of gametogenesis. As a result, instead of intact parental chromosomes, descendants obtain mosaic chromosomes, e.g. parts of paternal and maternal chromosomes 1 randomly combined into a new recombinant chromosome 1, different from both parental chromosomes 1. Thus, each chromosome is comprised of randomly combined parts of counterpart parental chromosomes. This is the next, mutational and recombinational level of stochastics. Due to the mutations and recombinations, the number of possible combinations of genes that can be transferred to descendants increases to 10 with many zeros. Of them, each descendant will get only one specific combination. And all this occurs at the level of chromosome assortment into gametes. Accordingly, formation of two identical individuals is impossible already at this level.

Level of development of an organism from a fertilized cell. Nonheritable mutational and epigenetic events. The above-mentioned monozygotic twins evolve when a single egg is fertilized to form one zygote, which then divides into two separate embryos followed by separate development of two organisms. The set of genes in the two initial cells is identical. However, even monozygotic twins are not identical. Their non-identity shows up at the next level of stochastic events, the level of recombinations and mutations that persistently occur throughout the entire period of development, in the course of division of all cells in our body, and also in germ cells. Assuming the rate of mutations at $\sim 10^{-9}$ per base pair and one cell division, even the first two cells generated by a dividing zygote differ from each other and from the initial zygote by a number of mutations. (Available estimates of mutation rates are very different, so I have used the value from two recent papers, i.e. [7, 8]). Each cell division is followed by new mutations randomly distributed in the genomes of newly formed cells. Therefore, each of us is a particular mosaic of 10^{14} somatic cells, each of which is slightly but still different from the other in genetic information. I suspect that the scale of somatic mosaicism is not fully realized. Although the fact of this phenomenon has been mentioned in such a classical work as [9], and also in some reviews (e.g. see [10]), it was usually considered in the context of mutations in certain loci [11-14] and, as a rule, in connection with obvious phenotypic effects, often pathologic. At the same time, a huge number of mutations stochastically arising across the whole genome either do not have any visible or have practically invisible phenotypic effects. However, all together they can form something like a genetic background that determines a unique expression mode of usually observable traits, in particular diseases.

Apart from mutations and recombinations, there is also the stochastics of epigenetic changes that acts at the level of organism development. These changes do not affect DNA sequence but are due to various chemical modifications of DNA, of which the most known is methylation. These modifications often affect regulation of gene activity, and their effect varies because of stochastics: a gene in one individual can be accidentally expressed at higher or lower level than its counterpart in another individual.

Epigenetic effects can be exemplified by isogenic viable yellow agouti (Avy) mice expressing various phenotypes. In these mice, a mobile element IAP is inserted into the genome at a distance of 100 kb from the specific promoters of the *agouti* gene that determines the color of fell. When IAP is inactive, the Agouti protein is specifically expressed in hair follicles at a definite stage of hair development, and the color of fell is agouti. In contrast, when IAP is active, the *agouti* gene is transcribed from the promoter of the IAP long terminal repeat (LTR), the program of the *agouti* gene expression is disturbed, and the Agouti protein is unspecifically expressed in all cells of the organism. The unspecific expression of the *agouti* gene results not only in the yellow color of fell, but also in obesity, diabetes, and increased frequency of tumors. This phenotype can stochastically variegate (be different in different cells) due to different methylation of the mobile element LTR in different cells. The methylated LTR is inactive, and its methylation in a given cell is a random (stochastic) event. In cells with the methylated LTRs, the *agouti* gene is regulated as in normal mice, and these cells give the hair color agouti, while in cells with unmethylated LTRs the *agouti* gene malfunctions and the color is yellow. As a result, these animals have spotted fells and are therefore epigenetic mosaics [15]. The authors of the original paper [16] write: "...we propose that stochastic and variegated silencing of transcriptionally competent retrotransposons makes every individual mammal a compound epigenetic mosaic. Active retrotransposons interfere with expression of genes around them; the unique epigenotype of individuals means that each will have a distinct pattern of interference and thus a different phenotype. This hypothesis specifically predicts that retrotransposon activity is controlled by cosuppression, that if expression of an individual retrotransposon can be assessed it will be variegated to a different extent and with a different pattern in different individuals, and that tran-

scriptional activity of retrotransposons will be found to perturb expression of genes in their vicinity. If epigenetics does have a significant impact on phenotype in mammals, it will present an interesting problem, as the rules governing this innate variation are likely to be completely different than those of Mendelian genetics." Having known that our genome is literally crammed with retrotransposons [17], and understanding that some of them can behave as discussed above, and similar effects can be provoked by not only retrotransposons, we can realize the scale of individual nonheritable epigenetic variability.

The level of organism development is also characterized by so-called stochastic noise. The molecules involved in gene regulation are often present in the cell in very low concentrations. This feature causes strong fluctuations in reaction rates among individual cells, which may in turn lead to serious deviations from normal development. In spite of that, development of animals is a very stable process due to so called canalization. In the course of their development, living systems are supposed to reproduce a stable phenotype against the background of rather appreciable fluctuations. Evolution has probably selected systems capable of resisting stochastic noise and rather accurately reproducing the parental phenotype. Canalization is a design principle wherein developmental pathways are stabilized to increase phenotypic reproducibility. It can be visualized as the process of formation of virtual "canals" in which developmental programs flow. The deeper the walls of these canals, the smaller the chances for the programs to deviate from the required trajectory [18, 19]. However, in spite of all "efforts", cells cannot prevent persistently happening stochastic events.

Finally, stochastic events must happen during formation of organs, for example the brain [20]. The human brain is an extremely complex structure. In the course of its development, a huge number of synapses (mostly chemical) are formed. Synapses are specialized junctions, through which neurons signal to each other and to non-neuronal cells such as those in muscles. They are crucial to the biological computations that underlie perception and thought. They allow the nervous system to integrate and control other systems of the body. The human brain contains about 10^{14} synapses (Wikipedia, http://en.wikipedia.org/wiki/Chemical_synapse). Would it be possible to imagine that such "microuniverses" can develop identically and without deviations in all cases?

"Stochasticity or the fatal "imperfection" of cloning" is the title of a paper published in 2005 [21]. In this paper, the author argues that it is impossible to create an exact copy of a living entity due to natural fluctuations in living systems that lead to mosaics in the course of development, which I tried to explain above. I would like to extend this idea and suggest that the probability of existing of two identical complex organisms is negligibly small even for one species both at each moment and in evolution.

The world of microorganisms is also extremely variable, but here can be exceptions to this "taboo" for individual microbial cells due to small genomes and therefore their smaller variability, as well as due to a huge number of microorganisms existing in nature. (Although who knows, if we take into account not only genetic variability but also stochastic variations in the number of gene expression products.) However, with a high degree of confidence it can be suggested that it would be highly improbable to find two identical clones (populations) of one microorganism, if these clones contain a sufficiently large number (10^8-10^9 or more) of descendant cells. Assuming the rate of mutations for microorganisms at $5 \cdot 10^{-3}$ per genome and per generation [22], an *Escherichia coli* clone of 10^8 cells, originated from a single cell in 16 h as a result of 32 generations (time of one generation is 0.5 h), will contain ~15% ($>1.5 \cdot 10^7$) mutant cells. These mutations in two clones under comparison will be partly the same but partly different. Thus, the law for multicellular organisms will be in this case the law for populations comprised of a multitude of unicellular organisms.

The taboo formulated above is an absolute law as well as the second law of thermodynamics. It is impossible to create perpetuum mobile, and it is likewise impossible to artificially create a copy of a multicellular organism or to find such a copy among ever existing multicellular organisms, be it human, goat, or centipede.

But will such a fundamental prohibition as "***Based on comparing modern organisms, it is impossible to restore the structure of the first living cell and the processes that have led to its emergence?***" be valid?

To make the sense of this taboo clearer, I will quote a recent paper [23], where the author writes: "Eukaryotic evolution is something of a Gordian knot. Using single genes to unravel it will not work, as the genomes of eukaryotes (animals, plants, fungi, and protista) are derived from those of several prokaryotes (eubacteria and archae). So the focus has shifted towards analyzing flows of gene populations, and even of entire prokaryote genomes, into eukaryotes. These more holistic studies are revealing the complex genetic and evolutionary connections between eukaryotes and prokaryotes.

More than two thirds of the nuclear genes of the yeast *Saccharomyces cerevisiae*, for instance, are derived from eubacteria, and the balance from archaebacteria. ...But how did evolution come up with the strange distribution of eubacterial and archaeal genes we see in eukaryotes today?

In prokaryotes, there are two major gene classes: operational and informational. Operational genes are involved mainly in day-to-day processes of cell maintenance, and code for amino acid and nucleotide biosynthesis as well as related functions (author's note: such as cofactors, components of cell membranes, intermediate metabolites, fatty acids, lipids etc.). Informational genes

feature primarily in transcription, protein synthesis, DNA replication, and other processes to convert information from DNA into proteins.

Because eukaryotes are derived from archaebacteria and eubacteria, one might expect to find an archaeal and a eubacterial copy of each nuclear gene. But strangely, archaebacterial operational and eubacterial informational genes are almost completely absent from eucaryotes. ...This is statistically an extremely unlikely event, and it needs to be explained. ...How the eukaryotic cell came to be is one of the greatest enigmas in biology. It is a story so complex that no single gene can tell it. Only entire genomes can." However, the available information indicates multiple losses and acquisitions of genomic fragments in evolution, and it raises doubts as to whether we will be able to understand what the first cells that have, in billions of years, given rise to present-day eukaryotes were. It is worth mentioning that the same is valid also for prokaryotic cells whose genomic lability and genetic losses and gains in evolution were repeatedly discussed (for review, see [24]).

The first cell might spring up, for example, from elementary ingredients under the action of a certain structure (I will call it "initiator") that was further discarded as useless. Since all descendant cells were produced by division, supporting the well-known principle "cell from cell" ("*Omnis cellula e cellula*"), there was no need in *de novo* assembly anymore. In evolution, there is a famous rule "Use it, or loose it". It seems, we will never know what was "initiator".

Such a prohibition would have an obvious consequence: ***it is impossible to create a living cell from its separate simple constituents.***

In 2002, the journal *Science* reported [25] that J. Craig Venter Jr. (a famous "sequencer" of the human genome) claimed he had won a state grant to construct a new form of life. The US Department of Energy awarded a US$ 3 million grant for three years to construct a synthetic chromosome, which would be the first step to a self-replicating organism with an entirely artificial chromosome. Actually, all this meant a synthetic mycobacterial genome with a minimal set of genes. The communication also contained a cautious concern about possible incompatibility of the synthetic chromosome with the organization of the future host cell. In the next year, the same journal published a more detailed explanation of Venter's purposes [26]. It is planned to first synthesize an artificial genome and then introduce it into a *Mycoplasma genitalium* cell with its own DNA somehow destroyed. What happens then is an enigma, says this communication. The genome may remain inert, unable to work. According to the author, such an approach is a paraphrase of the question "what is life?" in genomic terms.

I have little doubt that the synthetic *mycobacterial* genome will work in a *mycobacterial* cell, if this genome is error-free. But what is suggested is rather far from "creating altogether new, minimal genome organism" or creating the cell "from scratch", as it is termed in the popular literature. The genome is to be introduced into *an already existing, natural, assembled, and ready to work cellular environment* with its transcription and translation apparatuses, membrane structures, and many other attributes of the living cell. And this is very far from "starting from scratch". Indeed, recently the genome of *M. capricolum* was successfully replaced with that of *M. mycoides* to produce cells phenotypically identical to *M. mycoides* [27, 28]. This is a remarkable achievement having, however, nothing to do with the assembly of a cell from initial simple separate components. It may be added that an artificial minimal mycobacterial genome has already been synthesized [29], and I think that soon we will hear about the next success — the genome will work in a mycobacterium.

Actually, what is being done with microorganisms is a simplified variant of nuclear transfer from somatic cells into enucleated oocytes followed by development of viable progeny, provided that the transfer is intraspecies. Since the cloning of the world famous sheep Dolly, multiple successful cloning experiments have been reported. Moreover, in recent years, there appeared reports of successful interspecies transfer if the donor nuclei and recipient oocytes were taken from close species (for recent review, see [30]). Such a transfer was suggested as a means to rescue endangered species. However, the attempts of nuclear transfer between distant species have always met no success and resulted in developmental arrest of the interspecies zygote at very early stages of development. Constant failures of many such attempts [30] indicate the importance of the compatibility of the donor oocyte cytoplasm architecture and its other structures with the introduced foreign nuclear complex and all its structures.

Therefore, attempts to answer the question "what is life?" in this manner are still far from success and, to my mind, will never be successful due to high complexity of the system and ignorance of the "initiators" (see above) that have brought the cell to life in early evolution.

Having in mind all above considerations, I dare to formulate the last taboo: ***the mechanisms determining cell vitality are essentially incognizable***.

I will quote the idea of the prominent evolutionist Lewontin expressed in his book "The Genetic Basis of Evolutionary Change" [31]: "The subject of ... this book, the nature of genetic diversity among organisms, has always seemed the basic problem of evolutionary genetics. Because of the immense methodological difficulties and ambiguities, a characterization of that genetic variation seemed always to elude us. Then, with the discoveries of molecular biology about the relationship between genes and proteins, the possibility of an unambiguous characterization of genetic variation among individuals was opened. The first experiments revealed an extraordinary

wealth of genetic diversity and, quite naturally, those of us involved in the work felt an immense elation in having finally given a direct answer to the major problem that had been plaguing our field. ... As we tried to explain the great variation that had been observed, our original elation gave way slowly to disappointment. For no explanation is really satisfactory, and the kind of ambiguity that originally permeated the observations now pervades the theoretical explanations". Lewontin then formulates the problems that must be solved to assess the role and causes of genetic diversity, and further proceeds: "But such an assessment will depend on an understanding of the relation between gene and organism that far transcends any present knowledge of development, physiology, and behavior. In fact, it demands the answer to every other question that now lies open in biology".

This is the view of a leading biologist who solves problems in his branch of knowledge but does not forget general problems of biology that must be solved to get final answers to problems of particular biological branches of the knowledge tree. It implies a vicious circle: without having solved particular problems one cannot hope to solve a general problem, but neither particular fundamental problems of biology can be solved without having solved the general problem. If we are unable to understand the origin of the first cell and to assemble a living cell "from scratch", we will be also unable to answer the question "what is life?"

The biological taboos above restrict our capability of cognizing life and practical capacities. One cannot create an exact copy of a human being and to assemble a living cell from individual molecules or even from separate structures, one cannot know what the first cell looked like and why it has started to divide, etc. Is it bad or good for us? Neither this, nor that. This is just what should be remembered and what we should live with, as well as physicists live with the prohibition to simultaneously measure the coordinates and speed of the electron.

But let us suppose that we are restricted by the taboos formulated above, what does it mean for scientific research? "Abandon all hope, ye who enters here?" Pointless vanity? Surely not. The impossibility of "the final solution" does not preclude maximum approaching this solution, as well as the impossibility of creating a perpetuum mobile does not mean stopping the attempts to develop an engine with maximum efficiency. Such an approaching will in itself have enormous practical importance. It might bring medicine and biotechnology to a much higher level, improve quality of life, and solve many ecological and food problems. It is known that every success in fundamental biology has always led to much greater successes in application areas, including medicine and biotechnology.

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