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Prospects & Overviews

CBC-Clock Theory of Life – Integration of cellular circadian clocks and cellular sentience is essential for cognitive basis of life

František Baluška[1] | Arthur S. Reber[2]

[1] Institute of Cellular and Molecular Botany, University of Bonn, Bonn, Germany

[2] Department of Psychology, University of British Columbia, Vancouver, Canada

Correspondence
František Baluška, Institute of Cellular and Molecular Botany, University of Bonn, Bonn, Germany.
Email: baluska@uni-bonn.de, areber@brooklyn.cuny.edu

Abstract

Cellular circadian clocks represent ancient anticipatory systems which co-evolved with the first cells to safeguard their survival. Cyanobacteria represent one of the most ancient cells, having essentially invented photosynthesis together with redox-based cellular circadian clocks some 2.7 billion years ago. Bioelectricity phenomena, based on redox homeostasis associated electron transfers in membranes and within protein complexes inserted in excitable membranes, play important roles, not only in the cellular circadian clocks and in anesthetics-sensitive cellular sentience (awareness of environment), but also in the coupling of single cells into tissues and organs of unitary multicellular organisms. This integration of cellular circadian clocks with cellular basis of sentience is an essential feature of the cognitive CBC-Clock basis of cellular life.

KEYWORDS

cells, circadian clock, cognition, consciousness, evolution, redox homeostasis, sentience

INTRODUCTION

Circadian clocks are inherently cognitive in nature. They participate in a cell's sentient awareness of the environment by anticipation of the regularities in the surrounds such as gravity, light, and temperature which allow appropriate behavioral responses and safeguard organismal survival. Recent advances in our understanding of circadian clocks revealed their deep cellular basis.

As we have argued elsewhere, all known life is based on sentient cells.[1,2] The Cellular Basis of Consciousness (CBC)[1] model is grounded on the assumption that cellular life and consciousness are coterminous, that internal states, valenced experiences emerged with the first autonomous, self-replicating cell. The most important feature of the cellular state that supports these life processes is the plasma membrane separating the outside (non-life) from the inside (life). Besides allowing ordering of molecules (acting against the second law of thermodynamics) into biological macromolecules, the plasma membrane also represents a smart sensory border capable of handling energy and ion fluxes which enable its bioelectrical excitability. These dynamic fluxes allow cells to extract both energy and information from their environment, making them agents capable of acting in their own interests.[1,2] The processes that support cellular proto-cognition and nano-intentionality are based the membrane-generated bio-electro-magnetic cellular fields[3] acting as the cellular proto-consciousness.[1,4,5] Here we examine the role that temporal factors and natural cycles likely played in the formation of the first life-forms—in short, a "CBC circadian clock-theory" of life. Awareness of diverse cues from their environments was a central feature allowing the ancient vesicle-like proto-cells to evolve slowly into the first self-replicating and autonomous cells some 3.7 billion years ago.

CIRCADIAN CLOCKS AS ANTICIPATORY SYSTEMS EVOLVED IN ANCIENT CELLS

How cells evolved is unknown but it is logical to consider ancient proto-cells as a predecessor of the first self-replicating and autonomous

Abbreviations: CBC, cellular basis of consciousness; RC, redox cycle; SCN, suprachiasmatic nucleus; TTL, transcription-translation loop; TTR, transcription-translation circadian rhythm

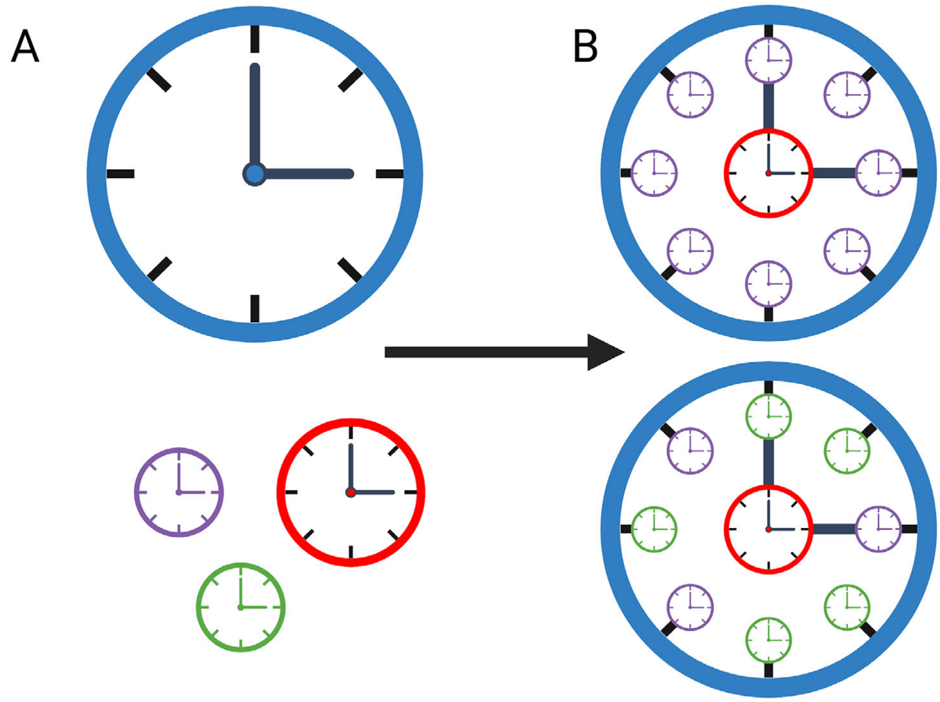

FIGURE 1 Cellular evolution of circadian clocks and CBC sentience. During some 4.5 billion of years (Ga) of cellular evolution, eukaryotic cells evolved from several prokaryotic cells, each endowed with its own version of a circadian clock and CBC sentience. Their integration into eukaryotic cells required co-ordination of their individual RC and TTL clocks (A). According to our version of the endosymbiotic theory,[16] the eukaryotic cell (in blue), its nucleus (red), mitochondria (purple), and plastids (green) each have their own semi-autonomous clocks; all of which are co-ordinated and integrated into the holobiont-like circadian clock of the whole eukaryotic cell (B). Similar integration of simple prokaryotic CBC sentience into the more complex CBC sentience of the eukaryotic cell can be envisioned

cells.[6] These proto-cells were represented by simple vesicles defining the outside and inside via lipid bilayers allowing their protection, energy extraction form their environments, and self-replication.[6–8] It can be expected that these proto-cells evolved an ancient version of circadian clocks at their limiting membranes, allowing them to anticipate changes in their physical environment and to evolve slowly into fully developed cells. Circadian clocks allowed survival of these proto-cells by being aware of fluctuations in their environment and to live long enough to evolve into the first full-blown cells. Cyanobacteria represent one of the most ancient prokaryotic organisms.[9–12] They are behind the atmospheric oxygenation known as the Great Oxidation Event some 2.3 billion years ago.[13] In order to survive, ancient as well as present circadian clocks had to have been coordinated and synchronized with the environmental time.[14] It is well known that circadian clocks are flexible and adapt effectively to change in the parameters of relevant environmental cues.

CELLULAR BASIS OF CONSCIOUSNESS AND BIOLOGICAL CLOCKS

The CBC model proposes that higher (supracellular) levels of consciousness are based on the single cell level of sentience.[1,2,4,5,15] In cellular evolution, the most difficult process was the assembly of the full-blown eukaryotic cell[5,16] which, while it may look simple since it was a symbiotic event involving two prokaryotes. However, it took approximately two billion years for the first successful eukaryotes to evolve.[6,16] Once stable eukaryotic cells were formed, the evolution of contemporary plants, animals and humans took about one billion years.

This process of biological evolution was shaped by physical cues with temporal features including the rotation of the Earth around its own axis every 24 h, the rotation of the Moon around the Earth, and the rotation of the Earth around the Sun. During cellular evolution, both physiology and behavior of cells have been shaped by predictable changes in the environment due to the Earth's rotation around its axis. Endosymbiosis-based evolution of the eukaryotic cells and true multicellularity both require functional integration of individual cellular clocks into supracellular clocks (Figure 1). We propose here that the development of the multi-cellularity of eukaryotic cells was a necessary consequence of integrated symbiotic supracellular clocks and sentience (Box 1, 2, Figure 1).

CIRCADIAN CLOCKS ARE BASED ON CELLULAR REDOX OSCILLATIONS

Circadian clocks of anucleate red blood cells[30,31] are based on redox homeostasis-based oscillations which have been highly conserved since their evolution in ancient proto-cells and first prokaryotic-type cells. For example, circadian clocks of cyanobacteria evolved some

BOX 1:
From cellular to organismal and social clocks

The simplest and most ancient circadian cellular clocks are found in cyanobacteria and other prokaryotes. More complex and compound circadian cellular clocks are found in eukaryotic cells where symbiotic partners have their own subcellular clocks and generate from them the circadian clocks of eukaryotic cells (Figure 1). There are two basic types of cellular clocks: the electronic redox cycles (RCs) and transcription-translation loops (TTLs). RCs are ancient and highly conserved, present already in prokaryotic cyanobacteria. They are based on oxidation-reduction cycles, driven by movable electrons, of peroxiredoxin proteins and numerous reactive oxygen and nitrogen species. RC clocks are independent of the nucleus and DNA-based genome. They emerged in ancient eukaryotic cells some 2.7 Ga after the cyanobacterial RC clocks. The genome-based TTLs are more variable with respect to their components. Both the RCs and TTLs are fully integrated into complex cellular circadian clocks of cells of contemporary multicellular organisms. Their cells integrate first into semi-autonomous tissue- and organ-specific clocks, which integrate further into the organismal clocks. In animals the so-called peripheral clocks are distinguished from the suprachiasmatic nucleus (SCN) of the brain's hypothalamus which represents the central clock (Figure 2). As with the cognitive functions, the biological clocks are centralized in humans and other animals and neurotransmitters are relevant in both situations. In plant bodies, each organ generates its own circadian clock based on integrating the cellular clocks. Importantly, plant cellular clocks are more complex than those in animals because of chloroplasts which run their own organellar clocks. Plant cellular clocks are coupled in tissue- and organ-specific manner, with cell-to-cell coupling of cellular clocks being the strongest in the root and shoot apices (Figure 2). Plant organs isolated from the plant body can maintain their organ-specific clocks as long as they can be kept alive.[17,18] Both animals and plants are complex holobionts and have large numbers of prokaryotes living with them, when the numbers of these prokaryotic cells can be even higher as the number of all their own eukaryotic cells.[19–21] Cellular circadian clocks of these prokaryotic and host cells are integrated into social supra-cellular clocks in a manner similar to the way the gut microbiome affects the brain and its cognitive and social activities. Social integration and synchronization of organismal clocks is also possible among individuals in social insects and mammals,[22–24] as well as between root clocks of plants and fungal clocks of symbiotic arbuscular-mycorrhizal fungi (Figure 2).[21,25]

BOX 2:
Cellular view of holobionts and hologenomes

The term holobiont was coined by Lynn Margulis in 1991 to denote sum of all cells of multicellular organisms and their microbiomes. Since then, the concept has been expanded and includes hologenomes viewed as the sum of all holobiont genomes.[25–28] In a strict sense, the eukaryotic cell, with all their symbiotic organelles,[16,19,26,29] is a holobiont (Figure 1). Eukaryotic cell hologenome is then the sum of the nuclear and all the organellar genomes. Intriguingly, it took about 2.5 billion of years for the eukaryotic cell to evolve from prokaryotic predecessors.[16,29] This suggests that the integration of cellular proto-consciousness and circadian clocks into the eukaryotic supra-cellular circadian clock was a most complex and difficult undertaking.

2.7 billion years ago.[32,33] Cyanobacterial circadian redox rhythms are based on electron transfer across their membranes, which can be detected and analyzed by extracellular electrodes.[34] This suggests that the first circadian clocks were ticking at the limiting plasma membranes of ancient cells and were connected to the extraction of energy from their environment through electrochemical redox homeostasis. This is based on electron transfers by electron donors and acceptors protein networks as well as across membranes.[34] This type of circadian clock can be seen in organelles of eukaryotic cells such as mitochondria and chloroplasts.[35–38] In contrast to transcription-translation circadian rhythms (TTRs), which are not deeply conserved across all three domains of life, the redox homeostasis circadian rhythms based on peroxiredoxins are highly conserved.[32] Obviously, the redox-based circadian rhythms are evolutionary older and upstream of the TTRs.[32,34,39–43] However, it can be expected that the genome-based TTR system and the membrane-based Redox-ROS systems are interconnected via multiple feed-forward and feedback loops.

INTEGRATED CELLULAR CIRCADIAN CLOCKS

The CBC model offers a bottom-up approach to the study of sentience and cognitive functions—which is a distinctly minority view within contemporary biopsychology. However, cells are the basic units of the living world, as atoms are the basic units of the physical world. At the cellular level, there is an absolute unity of life as can be seen in the topic of biological clocks which have a cellular basis in all organisms. For the endosymbiotic organelles, such as plastids and mitochondria, synchronization of their internal processes, including clocks, was central to achieve the full integration with their host cells. How this full integration of individual cellular clocks into the integrated supra-cellular clock of the eukaryotic cells was accomplished remains a mystery.

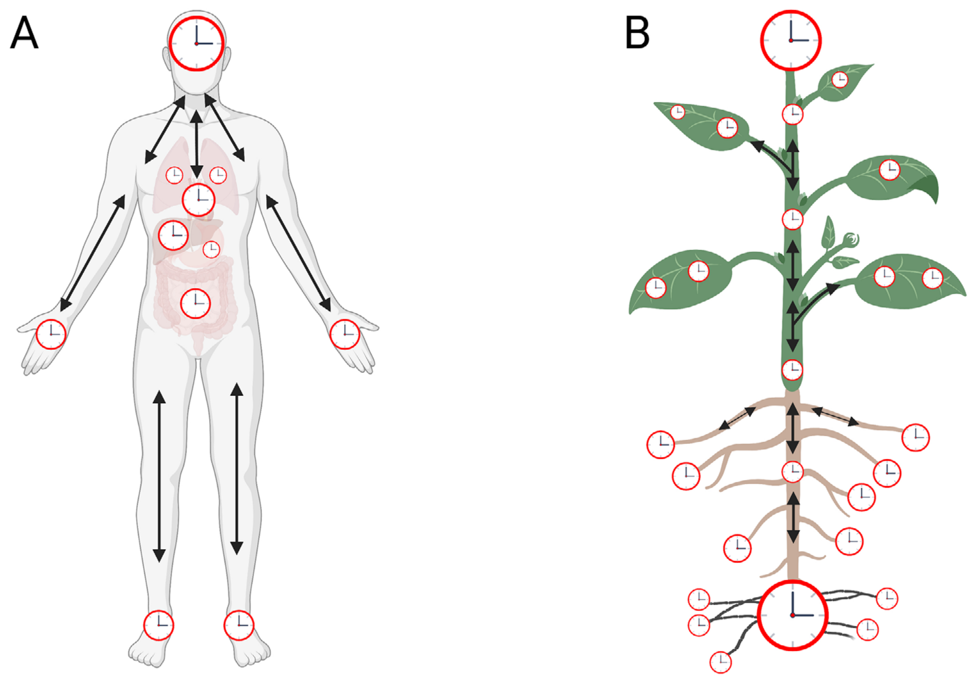

FIGURE 2 Organ- and tissue-specific circadian clocks in humans and plants. Schematic depiction of tissue- and organ-specific circadian clocks of humans and plants. These supracellular clocks are coordinated, by still not well understood cell-cell communication and integration processes, into supracellular circadian clocks. In humans, the SCN clock in brains represents the central clock integrating all the other peripheral clocks. In plants, the tightest coupling of cellular clocks is found at the shoot and root apex, whereas clocks of the vascular system (arrows) integrate all tissue-specific and organ-specific clocks into a unitary organismal clock. Root apices enjoy symbiotic interactions with arbuscular-mycorrhizal fungi, having their own fungal clocks. They all are presumably co-ordinated into the meta-holobiont circadian clocks.[109,115]

The original view of circadian clocks in humans and animals was that they are controlled by cerebral processes. However, more recent studies revealed that organs, tissues, and individual cells are running lower-level, semi-autonomous clocks based on individual cellular temporal mechanisms.[44–47] Symbiotic cell organelles, such as mitochondria and chloroplasts, are also running their clocks (Figure 1) which are integrated with the whole eukaryotic cell clocks.[48–51] In addition, the prokaryotic cells of the gut microbiome also generate and maintain their own circadian clocks.[52–54] The fact that circadian rhythms of the prokaryotic microbiome clocks synchronize the clocks of eukaryotic host cells suggests the existence of cell-to-cell synchronization, though the process remains unknown. The overall host physiology is integrated with its microbiome through coordinated efforts of individual cellular clocks.[55–59]

Circadian clocks based on redox oscillations are intact in anucleate mammalian cells such as red blood cells and platelets,[30,31,60] suggesting that the gene expression clocks are downstream of the plasma membrane-based clocks.[61,62] Similarly, as with organismal clocks, the animal and human gut microbiome (also termed the psychobiome) is known to affect the brain and its cognitive activities.[63–67] Interestingly, cancer cells break away from the host organism's circadian rhythms[68] while the aberrant circadian clocks can apparently induce cancer in host organisms.[68,69] Although it is still not understood how individual clocks harmonize together, communication and oscillatory coherence between the individual cellular clocks is accomplished through chemical neurotransmitter-like cell-to-cell communication.[59] In order to effectively integrate multiple cells into unison-ticking clocks, the ancient redox-homeostasis rhythms and the bioelectric phenomena at the excitable membranes are crucial. Both membrane bioelectricity[34,70] and reactive oxygen species signaling[34,70–73] are well suited for the effective cell-cell communication needed for coordination of all cellular clocks. Cells assemble semi-autonomous tissue-specific and organ-specific clocks (Box 1, Figure 2), all of which integrate into the organismal clocks, both in plants and animals.[44–47,59–61,74,62,75,76]

EUKARYOTIC CELL AS *CELLS WITHIN A CELL*: IMPLICATIONS FOR CELLULAR AND ORGANISMAL CLOCKS

In cellular evolution the most difficult achievement was the establishment of fully integrated eukaryotic cell which represents cells within a cell.[4,5,15,16,77–80] The process of symbiotic interactions of several prokaryotic cells, all of which had obviously their own cellular clocks (Figure 1), can be expected to require the integration of all these cellular clocks into the supracellular clock of the first eukaryotic cell (Figure 1, Box 1, 2). Similarly, in the CBC theory,[1] cellular proto-consciousness of all interacting cells was merging into a supracellular version of the cellular consciousness.[1,4,5] Eukaryotic cell is then a holobiont when its hologenome is the sum of the nuclear and all the organellar genomes (Box 2).

EXCITABLE PLASMA MEMBRANES AS MARKOV BLANKETS

Markov Blankets represent statistical tools that enable the study self-organized cognitive internal/external systems relevant for cells and living organisms. Excitable cellular membranes have several unique features which fulfil the properties of Markov Blankets [81–83] inherent for living nature of cells based on their cellular circadian clocks and consciousness. [4,5,84,85] The cell's plasma membrane provides a smart boundary condition which is the essential feature for life processes as the cell represents the first and primary niche construct in cellular evolution.[86]

There are fundamental similarities between collective supracellular timekeeping of circadian rhythms and the generation of organismal supracellular sentience through communicative entanglement of the individual cells.[1,4,5] Both the circadian clocks and cellular consciousness were integral features already at the very beginning of life (Box 1)[1,2] and are also features of supracellular clocks (Figures 1 and 2) and subjective experience.[87] We anticipate that future research will show that both are supported via the same processes based on the excitable membranes surrounding all cells, acting as Markov Blankets.[81–83] The CBC-Clock theory of life, in which cellular clocks and consciousness are closely interlinked (Boxes 1 and 2), is a framework for bringing us closer to understanding the biomolecular mystery of life.

Excitable plasma membrane-based Markov Blankets likely play a crucial role in the integration of individual prokaryotic cells into unitary eukaryotic cells, and in the integration of these eukaryotic cells into unitary supracellular eukaryotic organisms such as fungi, plants, and animals. As membranes of both symbiotic organelles and their host cells are hotspots for processes responsible for reactive oxygen species production, signaling and homeostasis; these excitable membrane-based Markov Blankets can be expected to be inherently connected to the cellular circadian clocks.

ANAESTHETICS AND CELLULAR CIRCADIAN CLOCKS

Both the cellular circadian clocks and CBC sentience are based on the excitable membranes.[2–5] In our proposal, the CBC-Clock theory of life postulates that cellular circadian clocks should be sensitive to experimental treatments that compromise sentience. In fact, isoflurane-induced general anesthesia shifts the circadian clock in a time-dependent fashion in diverse organisms.[88–92] Interestingly, anesthetics were reported to induce loss of sensory-based organ movements and of ROS homeostasis both in animals and plants.[93–97] The phenomenon of general anesthesia is found not only in humans and animals, but also in plants, protists, and prokaryotic organisms.[98–100] In words of Claude Bernard "… what is alive must sense and can be anaesthetized, the rest is dead."[97,98]

Importantly, general anesthesia in Drosophila was reported to be related to electron spin[101,102] and xenon isotopes with nuclear spin are less potent in their anesthetic actions as than those without nuclear spin.[103] Moreover, xenon-based anesthesia includes also electron transfers involving the radical pair electrons.[104] Xenon is one of the most potent anesthetics, effective on both animals and plants. As xenon is chemically inert, its anesthetic actions on both animals and plants suggest that the clues for anesthesia and consciousness should be sought at the border of biology and physics, perhaps very close to quantum physics phenomena.[103–106]

As both the redox-based circadian clocks and anesthetics-induced loss of sentience are closely related to electron transfers and ROS homeostasis, it can be expected that these elusive phenomena will be closely linked. The cognitive aspects of circadian clocks, allowing cellular and organismal survival in the fluctuating environment, as well as environmental awareness via the CBC theory[1–5,14] provides us with a foundation for the novel CBC-clock theory of life.

THE NECESSITY OF SENTIENCE AND CLOCKS FOR LIFE

There is an aspect of the primordial world that is oft-neglected in discussions of the origins of life and, by extension, the origins of sentience. Variability dominated, change was everywhere, temperatures shifted, nutrient concentrations waxed and waned, acid concentrations came and went, toxic substances put in appearances, light levels shifted daily and seasonally, temperature gradients followed irregular patterns. From an adaptationist point of view, it is hard to imagine a non-sentient life form having any chance of emerging, let alone surviving, thriving and becoming the organic foundation for all the life forms, extant and extinct that followed. A prokaryote without sentience, one lacking valenced perceptions, one bereft of organic clocks, one that did not know which molecules to admit and which to block, would have been a Darwinian dead-end.

In his recent book Dennett,[107] in an effort to argue against this fundamental principle, distinguished between "competence" and "comprehension." Primitive species, including the unicellular bacteria of the book's title, have the former but lack the latter. They function effectively but do so without any subjective experiences, without sentience and without any internal representations of their actions. Such a framework, despite Dennett's creative efforts, makes little sense.

Such robotic functions and processes would have to have been based on genetic processes that operated independent of feelings, of subjective awareness, sentience. The obvious problem is, given the vast array of functions exhibited by prokaryotes, there would have had to have been a remarkable array of genes, each linked with one or more processes including monitoring temperature, nutrient levels, light levels, gravity, diurnal cycles, acid levels, communication, cooperation, learning, memory, decision-making functions, all of which are well documented.[1,2,4,5,15] Critically, all would have to have been operating semi-independently of each other. It is not only unclear how such a mosaic of DNA-based systems could have evolved, it is unlikely in the extreme. Far more likely is the emergence of a valence-based sentience that monitors internal conditions, evaluates the external environment, and achieves homeostasis by controlling cell membrane permeability and excitability.

Dennett, of course, is not alone. This notion that consciousness, sentience only appeared with the evolution of more complex species, ones with nervous systems, is still the dominant view in evolutionary biology. But, from the perspective of our CBC-Clock model, it is clear that it actually has little explanatory power. The nature of sentient minds did, of course, change as more complex species evolved and the emergence of neural circuits allowed for more sophisticated forms of mental life, but it did not start there. It is an inherent aspect of life, all life as we know it. As we have argued elsewhere,[1,2,4,5,15] life began once and it was cellular sentient life.

OUTLOOK

Besides the cellular circadian clocks, electron transport and ROS homeostasis are also central features in both photosynthesis and aerobic respiration.[5,108] Interestingly, as with anesthesia, the process of photosynthesis is located at the border of classical and quantum mechanics where vibronic couplings steer the molecular energy transfers via redox-based cyclic electron transfers at photosynthetic reaction centers.[109–112] As photosynthesis was invented by ancient cyanobacteria, together with the redox-based circadian clocks, some 2.7 billion years ago,[32,33] a logical entailment is that all three phenomena (circadian clocks, sentience, and membrane-based electron transfers) are inherently interlinked. The picture gradually emerging is that life, as we know it, is inherently bioelectronic and that the genome and proteome need to be completed by the senome and electrome.[5,70,113–119]

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CONFLICT OF INTEREST

There are no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

Data sharing not applicable–no new data generated.

ORCID

František Baluška https://orcid.org/0000-0001-8763-7861

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