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COMPLEX MULTICELLULAR FUNCTIONS AT A UNICELLULAR EUKARYOTE LEVEL: LEARNING, MEMORY, AND IMMUNITY

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According to experimental data, eukaryote unicellulars are able to learn, have immunity and memory. Learning is carried out in a very primitive form, and the memory is not neural but an epigenetic one. However, this epigenetic memory, which is well justified by the presence and manifestation of hormonal imprinting, is strong and permanent in the life of cell and also in its progenies. This memory is epigenetically executed by the alteration and fixation of methylation pattern of genes without changes in base sequences. The immunity of unicellulars is based on self/non-self discrimination, which leads to the destruction of non-self invaders and utilization of them as nourishment (by phagocytosis). The tools of learning, memory, and immunity of unicellulars are uniformly found in plasma membrane receptors, which formed under the effect of dynamic receptor pattern generation, suggested by Koch et al., and this is the basis of hormonal imprinting, by which the encounter between a chemical substance and the cell is specifically memorized. The receptors and imprinting are also used in the later steps of evolution up to mammals (including man) in each mentioned functions. This means that learning, memory, and immunity can be deduced to a unicellular eukaryote level.

Keywords: learning, memory, immunity, unicellular, receptor memory, hormonal imprinting

Introduction

In a multicellular organism, e.g., in a vertebrate or mammal, different cell types are in the service of different functions and these cells bunched together to form organs or systems. A unicellular is a complete organism in one cell, which has to fulfill all of the functions that are required for its primitive life. This can limit the functions that are used by it, or the functions believed to be present only in

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higher ranked animals are also working in this level, providing the evolutionary basis of further development. Considering this latter possibility, it seems to be worth to study the facts, which support or refute the presence and working of three complex multicellular functions (learning, memory, and immunity) in unicellulars.

Facts

Immunity of unicellulars

Immunity is a defense mechanism which protects an organism from pathogens attacking from outside as well, as developing inside (e.g., cancer). However, not only complete multicellular organisms have the immune abilities but also single-celled organisms like unicellular animals (protozoan) as well. Two types of immunities are known: the adaptive immunity working only in jaw animals and innate immunity working in all the animals from the unicellulars to the man. The ancient immunity is the innate one to which the adaptive immunity is joining later. The more primitive immunity is the innate, which is not able to differentiate individually; however, the adaptive immunity is not working without the innate one [1, 2]. Both types of immunities are based on the self/non-self discrimination. As the adaptive immunity appeared about 500 million years ago, in the case of unicellulars, only the innate immunity can be present [3], although there are different opinions [4–6]. However, the presence of a special form of innate immunity is absolutely required for the life of unicellulars, without which this grade of animal evolution would not be possible [7], and from the immunity present already at unicellular level evolved the more developed adaptive/innate immunity, characteristic to vertebrates [4, 8].

Self/non-self differentiation by immunity means that the cells are recognized and handled differently. For the recognition, markers are required, which are embedded in the plasma membrane and also receptors that can recognize the markers. If the marker indicates the selfness, the cell bearing the marker will not be attacked, or engulfed by phagocytosis and without this mechanism, the cells "consume itself and vanish from the face of the Earth" [9]. Simultaneously, the presence of the marker signals the biological possibility of pairing (difference in mating types), which is required for the propagation of the species [10]. However, using this mechanism, there is not a possibility of individual differentiation, for which the fine distinctions between closely related structures would be required [11], and which is present in the case of adaptive immunity [12]. This type of (innate) immunity is enough for protecting the unicellulars from the attack of viral or other infections, but there is not an immunological memory present, which could help to avoid reinfection and is very important in case of adaptive immunity.

Phagocytosis

In some opinions, unicellulars have more type of reactions for defending themselves [8]; however, phagocytosis is the outstanding tool for demolishing non-self attackers. The engulfment also has nutritional function: the engulfed material (cell) is digested and used for feeding the predator cell. This mechanism, which already appears at unicellular level, is persisting up to the highest level of evolution with similar manner serving the functions of defense and nourishment.

The amoeba *Hartmannella* catches invading bacteria (non-self cells) aggregating in the surface, as there is no separated place for recognition in the plasma membrane, and from any place, digestive vacuoles can be formed [13, 14]. This means that non-self is recognized and annihilated by unicellulars. In addition, the amoeba can discriminate between microorganisms to be engulfed: the similar flagellates, *Monas* and *Chilomonas* sp., which are similar in shape, are discerned by *Amoeba proteus*: 100 times more *Chilomonas* are engulfed than *Monas* [15]. The discrimination capacity is also very high at intracellular level: if a nucleus is transplanted between the individual of the same species (*Amoeba discoides* or *A. proteus*), viable clones can be produced; however, in the case of xenografts, the cells die [16]. A higher selectivity can be observed in ciliates: *Paramecium bursaria* can differentiate between organic particles by size and nature [17] and also in stentor, where organelle transplants of the same species produce viable chimeras, whereas, in case of xenotransplantation, the cells degenerate or die [18]. The old data mean that there is an exact – not individual – recognition of non-self at a unicellular level. Fresh data show that the sentinel cells of the social amoeba *Dictyostelium discoideum* (living already about 1.3 billion years ago) produce extracellular traps killing slug-invading bacteria as it is performed by neutrophils, as an innate immune defense mechanism [19].

Hormonal regulation of phagocytosis at a unicellular level? The aforementioned data show that phagocytosis is working at a unicellular level similar to, e.g., mammalian phagocytosis [20]. This means that phagocytosis is a well-conserved mechanism, which serves immunity, as well as nourishing; however, its role in the reproduction of cells has been lost. In multicellulars, phagocytosis is influenced by hormones (e.g., by histamine and serotonin), and this type of regulation can also be deduced to unicellular level [16]. As unicellulars are producing, storing, and secreting a lot of hormones, these can also participate in the regulation of phagocytosis [21, 22].

The cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) system is functioning in *Tetrahymena* and phagocytic activity is influenced by them [23]. Hormones acting by the transmission of these mechanisms influence phagocytosis [11, 16, 24], and these hormones could be

so simple as histamine and serotonin as well, as so complicated as insulin or adrenocorticotropic hormone (ACTH) [25–29], and sometimes, they influence the effect of each other [26]. There is also an opioid mechanism (in *Tetrahymena*), which means that the unicell synthesizes opioids and has receptors for them, modulating phagocytosis [30–32]. The unicellulars have receptors for the target of phagocytosis as well, as for the hormones regulating the process.

Learning and memory in unicellulars

Although they are interacting, learning and memory are different. While learning is an active behavior, memory is stable. Learning is a process by which a cell or an organism collects knowledge, whereas the memory stores them. The learning is a short-term activity, memory is long, and however, there are two types of it: short-term- and long-term memory. Short-term memory has a limited capacity for memorizing and the briefly stored material shortly disappear. Long-term memory can store information lifelong in case of occasional refreshments. In the case of unicellulars, there is a possibility of learning and as a consequence of it, the storage of the information (memory), is also possible.

Learning. The study of learning at a unicellular eukaryote level is controversial. Primitive forms of learning were studied and although the results were clear, the explanation of them were problematic. In case of *Paramecium*, the rapid increase of tube-escape learning was demonstrated after exercise [33]; however, it was not believed as associative learning [34]. Nevertheless, in similar experiments when retreat from the dead end of a long capillary which was too narrow for turning, a long-term backward swimming developed, which lasted 5 to 10 times longer [35], and this rather developed after exercise. In other experiments, brightness discrimination was associated with electric shock [36] and the results indicated that learning is possible. In *Paramecium*, classical conditioning was performed, when vibration and electric shock were coupled, which resulted in a lifelong sensitization [37, 38]. Simultaneously, conditioning to light shock was unsuccessful [39]. In the last time, habituation was studied in *Physarum polycephalum*. Bitter substances, quinine or caffeine, were used in the experiments, and after some repetitions, the unicell has been insensible to them [40]. This behavior is characteristic to habituation, which is a primitive form of learning, and demonstrates indeed that learning can be formed by non-neural cells in such a single-celled organism.

Memory. As early as 1905, Jennings [41] mentioned in his book that *Paramecium* has a primitive kind of learning and memory. Since this time and especially in the present days, many new data support this, and more sophisticated experiments are demonstrating. The myxomycete *P. polycephalum* is able to balance

their own diet [42], and has a spatial memory, which helps its ability to navigate in complex environments [43] and in the anticipation of the next stimulus to arrive [44].The ciliate *Tetrahymena* memorizes the geometry of a swimming arena (water droplet) [45], and its short-term memory helps its migration toward an attractant [46]. In chemotaxis experiments, the social amoeba *Dictyostelium* also exhibits memory [47], which helps in the migration over large distances.

Receptor memory and hormonal imprinting

The unicellular ciliate *Tetrahymena* synthesizes hormones characteristic to higher vertebrates [48–53] and reacts to them [54–59]. It has receptors, similar to mammalian ones, in the ciliary membrane [60–63], and has the transmission systems as well, with the transmitter materials, e.g., cyclic AMP and GMP, calcium–calmodulin, inositol phosphates, etc. [64–67]. This means that it has a hormonal system, which can react to external stimuli, and can communicate with the help of chemical messengers [57]. This system is chemically and functionally similar to that of mammalian ones. The hormone concentration required for communication is extremely low, which adapts to the watery life form of *Tetrahymena*. The first encounter between a hormone and the plasma membrane of *Tetrahymena* provokes the phenomenon of hormonal imprinting, causing the stabilization of receptor in the membrane and the receptor memory. As a result of imprinting, the reaction to the hormone in subsequent cases is changed, usually enhanced. The change is prolonged up to the 1000^{th} generations [68–71], or one year if the cell does not divide [72]. This means that the effect of imprinting is fixed in the imprinted cells; however, it is transmitted between cell generations, without alterations in base sequences (epigenetic inheritance) [73].

The life functions of *Tetrahymena* are influenced by hormonal imprinting; however, the receptor memory is inclined to slightly decrease after many generations. It is especially interesting that toxic substances in toxic dose can provoke imprinting, which protects later to the effects of imprinter [74]. Such a low dose of 10–18 M, which at the first encounter does not influence the function of the cell, can provoke long-lasting imprinting [75]. However, higher level of the imprinter provokes firmer imprinting and greater response on reexposure. The length of imprinting is also a very important factor. Cell growth inhibitors and material that inhibits cluster formation in the plasma membrane reduce the execution of hormonal imprinting [76]. Foreign signal molecules present just after hormonal imprinting can disturb the process (retroactive interference) [77].

The effect of a hormone to the unicell seems to be specific in many cases [78]. Insulin influences glucose uptake and also stimulates cell division in

Tetrahymena [79, 80], and imprinting can be produced [81]. Inversely, glucose influences insulin production [82]. Histamine increases phagocytosis and the phagocytic capacity is extremely elevated after imprinting [83]. There are some species differences in the imprintability [84].

Conclusions

The observations and experimental data listed above show that learning, memory, and immunity can be found in such a low evolutionary level, as in unicellulars. The life functions, the presence of which is conventional in a higher level of evolution, can be manifested in similar or different form related to the higher level (multicellular) habit; however, in most of the cases, they represent a basic form which is involved in the more complicated systems of multicellulars.

Man is inclined to use an anthropocentric approach looking after human or mammalian characteristics in lower steps of evolution. However, inversely, life characteristics of lower phylogenetic grades must be found at a higher level. Comparing the cellular functions between the unicellular life form and a cell in the multicellular, e.g., mammalian organism, there are many similarities and differences. There are such functions which are performed at the same manner, whereas others are very different. Phagocytosis is perhaps the best example of a function executed similarly in unicells and, e.g., in human macrophages; however, the goal is similar and different alike. Both cells recognize bacteria and engulf them; however, this is a main form of defense and nourishment for unicell itself and only defense of the organism in multicellulars, without the importance from nourishing aspects. This means that an exclusive cell function of unicells became a part of a multicellular (immune) system, which uses it in the same form, as already observed at a unicellular level. There is a similar situation in the case of hormonal imprinting, when the memorization of the first encounter with a signal molecule imprints the cell for the change of reaction for the individual life of the cell, and its consecutive progenies in the organism.

Why hormonal imprinting is beneficial for the unicells? The molecules which are named hormones in multicellular animals are tools for transmitting information from a place of an organism to another one. In this aspect, the use of the name hormone is meaningless in a unicellular organism. However, these molecules are able to transfer information between the individual unicells and organize them to a unified community, which behaves similarly. After imprinting, the cell can recognize molecules suitable for feeding or poisoning and reaching them or escaping from them much easier than without it. For sustaining the population, this is absolutely required for which the development of such type of

memory was necessary. In addition, the individual life of unicell is short; consequently, the effect of imprinting has to be transmitted to the progeny generations. As hormonal imprinting was fulfilled as a primitive form of memory, it subsisted in higher ranked animals in a similar form. The important difference is the developmental period of its formation: whereas in unicells, imprinting can be provoked in any phases of cell life, in multicellulars (e.g., in the mammals), the perinatal period or the differentiation of cells is suitable to create it [85]. As it was mentioned, the life span of a mammalian organism is long, whereas that of unicell is short. In any phases of life, the unicell can be imprinted, as the effect of the process will be manifested in the progenies; however, the age of cell culture is slightly influencing the effect [86]. There is no differentiation of cells; however, there is a fast multiplication with the same characteristics in the progenies. In the case of mammals, the matured progeny generations of the imprinted cells also utilize the effect of imprinting. The process and result are identical in both cases.

Neural memory is not required for hormonal imprinting in *Tetrahymena* as well, as in rat. In case of *Tetrahymena*, the single cell is responsible for the acceptance of the imprinter and for the execution of its effect. In case of the mammals, the cells isolated from the organism can be imprinted as well as inside the organism. The memory is present at the lowest phylogenetic level and also manifested at a mammalian level. In addition, cells of the central nervous system can be similarly imprinted, as blood borne isolated immune cells [87–89]. The mechanism is conserved during the million years of evolution, similar to phagocytosis.

Accepting that there is a primitive memory at the phylogenetic level of unicellulars, the most important question is: what could be the site and mechanism of the memory. In the case of higher ranked animals (vertebrates and mammals), the central nervous system memorizes in different sites of it. However, in a unicellular organism, only one cell is at the disposal of the process. Simultaneously, this cell must react to more different impulses than a specialized cell in mammals. A unicellular, which is living in a watery milieu, has to respond to numerous different chemical and physical stimuli, and also has to recognize similar (self) or foreign unicellulars simultaneously or in consecutive time periods. And, considering the results of hormonal imprinting, it is able to memorize them. What would be the tools of this process at a single-cell level?

In higher ranked animals, the cells have receptors for recognition of different molecules. These receptors are genetically determined and inserted into the plasma membrane (or cytoplasm, nucleus), pointed just to the molecule, which is important for the organism. For example, insulin receptors of the liver cells can recognize insulin, and thyrotropic hormone (TSH) receptors of the thyroid can recognize thyrotropin. The hormones and their receptors are determined at gene

level, and also the first encounter of them in normal (physiological) cases. However, in the case of a unicellular living in a pond, the number of molecules which has to be recognized is endless and the first encounter cannot be forecasted.

It can be hypothetically surmised that the cell membranes of unicellulars are dynamic structures, the components of which are continuously changing [90–92]. During their transformation, such configurations are formed which can fulfill the structure and function of receptors. These temporary "receptors" continuously control the molecules in their neighborhood and inform the cell about their presence. If there is a signal molecule among them, the receptor-like membrane structure is stabilizing and forms a real receptor, the binding capacity of which is durably increased. This could be the mechanism of hormonal imprinting, at least at a unicellular level. If the suitable structure (which is able to bind the stimulating agent or to react on it) is present in the plasma membrane, the cell reacts to the signal; however, after imprinting, this reaction is altered (will be stronger or weaker) and can also be manifested in the case of the mentioned learning in the presence of some chemicals (e.g., insensibility to quinine or caffeine after repetition [40]).

What could be the origin of self/non-self recognition in unicellulars? In *Tetrhymena*, amino acid recognizing receptors [93] must be present in the plasma membrane, as they are required for the feeding of the unicellular. As Lenhoff et al. [94] described, "such receptors (may be involved in chemoreception, neurotransmission, and hormonal activation) evolved in higher form from primitive receptors which responded to a broad spectrum of amino acids and peptides and which originally functioned in cell drinking and eating." These are not temporary but permanent components of the membrane. From these receptors, under the pressure of signal molecules (imprinting), hormone receptors can be developed. These amino acid receptors can bunch together temporarily in the presence of a foreign protein, forming non-self-recognizing receptors without individual specificity.

There are theories proposing that all living organisms must express both innate and adaptive immunities. This cannot be valid in the case of unicellular organisms, as adaptive immunity requests multicellularity. However, innate immunity is coded inside the genome; so, it can be the property of unicellulars. This also means that it is impossible to speak on immune "system" in unicellulars, as a system requires the presence of multicellularity. In unicellulars could be immune receptors which can discriminate between self and non-self universally, which diversificate, when adaptive immune system – which is the adaptation of the highly evolved unicellular immunity [4] – appears. This "newly evolved defense mechanism did not replace the previous one, but supplemented it, resulting in a layered structure of the immune system" [12].

The neural system (learning and memory) and the immune system (self/non-self) are the recognizing systems of the higher ranked animals. Both have similar capacities: to recognize inner and outer impulses and store the knowledge in the (immune or brain) memory [95]. Both are able to learn and remember the learned events and data. Both have the ancestor at unicellular level in the form of plasma membrane receptors. These receptors can react to chemical and mechanical impulses as well, as size and form. These receptors originated from food receptors, which are absolutely required for the sustenance of life also in such a low level and the receptor property remains during the whole evolution, as receptors are also used by the nervous and immune systems alike. However, at unicellular level, there is no distribution of functions between the cells, as there is not a complex cognitive network, as in higher ranked animals. Here, one cell fulfills all of the functions and perhaps the same receptor components are participating in the whole recognizing process, manifesting variations with the help of dynamic receptor pattern generation (formation of pattern-recognizing receptors). It seems to be likely, that some imprinting processes help the fixation of the suitable receptors and by this the specification of the cellular response and memory. This means that learning, memory, and immunity are present at a unicellular level in a primitive form, which is the basis of the higher ranked neural and immunological functions, and the primitive tools (receptors) preserve their importance in them. This also means that learning, memory, and immunity can be deduced to a unicellular level, where these functions show some similarities.

It is believed that the specific memory to a given stimulus is the property of vertebrates and is absent in plants and invertebrates, including unicellulars [95]. However, it is far from reality. Although this is not a neural memory, but an epigenetic one, the hormonal imprinting (in unicellulars) justifies that they can memorize a chemical stimulus. This epigenetic memory also has an important role in the long-life memory of the vertebrates [96, 97], and hormonal imprinting in unicellulars demonstrates that it can be deduced to unicellular level and justifies that memory does not request the presence of a neural network. The hormonal imprinting is an epigenetic process that inherited to the progenies without the alteration of base sequences, or permanently remains in the imprinted cells [98] and in their progenies. This epigenetic memory also has an important role in the case of innate memory in general [99]. This is frequently named as trained immunity [100], and gene expression is influenced by it. It does not require the participation of nervous system, as it is a typical cellular memory and consequently [101] it can be used by such single cells like unicellular animals or macrophages [102]. It uses the alteration of methylation pattern of genes, as a tool. The epigenetic memory has an important role in the life of unicellulars as their enemies – viruses and bacteria – can induce epigenetic alterations

(reprogramming), which are inherited to the progenies and help to vanquish further infections [103, 104], and also helps to adapt to environmental changes [105]. As the inherited epigenetic alteration is not a genetic (base-sequence dependent) one, it introduces a Lamarckian factor into evolution [106]. Its transmission is ubiquitous [107] and selection-based in unicellulars [106–109].

Summarizing the results of the study, it can be declared that the three functions written in the title of the paper: learning, memory, and immunity can be found in unicellular eukaryotes. The mechanism and execution of the functions are more primitive than that of the vertebrates; however, these primitive mechanisms provide the basis of higher level ones and allow to postulate the origin of these functions from that of the unicellular level [110, 111]. The immunity of unicellulars corresponds to the criteria of innate immunity. The vertebrate hormones affect *Tetrahymena* in extremely low concentrations [112] and hormonal imprinting justifies the presence of a non-neural memory, which is an epigenetic one and completely models the hormonal imprinting in mammals [24, 113]. There are very scarce data on the capacity and mechanisms of learning in unicellulars; however, the data which are at our disposal support the presence of a non-neural ability [114, 115]. While innate-like immunity of unicellulars does not contain the function of immunological memory, hormonal imprinting shows that the epigenetic memory is working at this phylogenetic level [116] and makes possible the transmission of the non-neural imprinting caused by a single encounter for an artificially evoked very long period of individual life [117] or the transmission of memorized information across many generations [118, 119].

Conflict of Interest

The author declares no conflict of interest.

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