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SOME CONSIDERATIONS ON THE TRANSITION FROM UNICELLULAR TO MULTICELLULAR LIFE

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Abstract: The transition from unicellular to multicellular life is estimated to have occurred dozens of times in the history of evolution. This paper discusses the results of two recent investigations: Prochnic's investigation (2010) on *Chlamydomonas* and *Volvox* and Anderson's et al. investigation (2016) on the molecular phylogeny of GK_{PID} , which show that the transition from unicellular to multicellular state did not require major genetic restructuring. At times even point mutations resulted in important consequences for the function of some proteins in living organisms. Other molecular mechanisms that contributed to the evolution and the complexity of the living world are also being discussed.

INTRODUCTION

The emergence of eukaryotic multicellular organisms and their diversification was one of the most important events in the history of bioevolution. Eight „major revolutionary transitions” with regard to increases in biological complexity (9, 18) have been identified in the process of evolution of the living world; they involved changes in the replicating units, in the way information is stored and/or transmitted and also in the division of labour. These transitions include the origins of some phenomena and biological events such as: the first cells, the genomes, the genetic code, the eukaryotes, the sex, the differentiated multicellular organisms, the colonial super-organisms and the language. Maynard Smith and Szathmáry, (1995), and Heron (2016) consider that multicellularity was one of the most critical evolutionary innovations.

The diversification of life and the hierarchical organization of the living world are also in Michod's opinion the consequences of a series of transitions: from genes and gene networks to the first cell, from prokaryotic to eukaryotic cells, from unicellular to multicellular organisms, from asexual to sexual populations, and from solitary to social organisms – transitions that required the reorganization of fitness (the transfer of fitness from a lower-level individual to a higher level). In the author's opinion, the evolution of multicellular organisms is an example: from lower-level individuals (cells) into a higher level individuals (multicellular), (20).

It is considered that the transition from unicellular to multicellular organisms has occurred dozens of times within the evolutionary tree during time, despite not having solid proof of it due to extinction or lack of transitional fossils (9). Another interesting aspect is that multicellularity has appeared independently in clades. When seen simply as cellular aggregation into multicellular organisms, the event is estimated to have occurred more than 25 times. Complex eukaryotes are believed to have evolved 8 times: once in the Animalia, three times in the Fungi and six times in three major plant clades (22). Transition in this type of organisms involved the development of some mechanisms of adhesion, recognition, cooperation and communication between cells (with processes like cell signalling, cell proliferation and cell survival also implied) and their association in tissues, organs and precisely coordinated systems. As natural selection acts on phenotypes, it promoted cell aggregations and consequently combinations of traits, being more functional, with better chance at survival and capable of producing more offspring than the unicellular counterparts they derived from, (23, 29, 40). It is obvious that the road from unicellular organisms 3 billion years ago to complex organisms, such as man (with over 200 different types of cells in the body)

was „such a long road", paved most probably with both failure and success, a road we are trying to decipher today using the modern investigation techniques at our disposal.

Hypothesis of the transition from unicellular to multicellular organisms

Three hypotheses have been developed in this respect (17, 28):
a) *Symbiotic hypothesis* - multicellular organisms occurred from symbiosis of different species of single-cell organisms; it is based on symbioses still present today between certain organisms (mutualism: clownfish and sea anemone; protozoans and termites; spider crabs and algae; microbiota in human digestive tract, etc.). The hypothesis has its roots in the *serial endosymbiotic theory* concerning the origin of eukaryotic cell (7), largely accepted by evolutionary biologists, and which by extrapolation was meant to explain the appearance of multicellularity through successive symbioses, a hypothesis that has not been accepted as valid, though;
b) *Syncytial hypothesis (of cellularization)* - multicellular organisms may have evolved from multinucleate unicellular organisms in which an internal membrane may have compartmentalized the cell into several. However, this hypothesis did not gather supporters, either, since it is unlikely for such an organism to have one main nucleus and many micronuclei used for reproduction, (17);
c) *Colonial hypothesis* - multicellular organisms may have occurred from symbiosis between unicellular organisms of the same species, when cells fail to separate following division. This hypothesis was first proposed by Ernst Haeckel in 1874 and it seems to be the most plausible of the three, having the most supporters.

Some characteristics and differences between unicellular and multicellular organisms

In a synthesis article, Niklas and Newman (2013) showed that multicellularity can be either „simple' – when the cells remain in contact with the external environment, or „complex" – when the cells lose this contact, leading to internalization. Differentiation in multicellular organisms is based on some of their characteristics: a) differences in cell specialization, energy consumption/expressed gene, increases in the amount of repetitive DNA (that do not encode proteins); b) the probability for a multicellular organism to return to unicellular state; c) the possibility to estimate when a simple multicellular organism reaches a size or morphology that necessitates specialized tissues for the transport of nutrients (22).

Multicellularity allowed greater size and complexity of organisms, differentiation from numerous cell lineages, coordinated cell division, and sexual reproduction. However, unicellularity was successfully, considering the fact that unicellular organisms are more abundant on Earth than multicellular organisms and have been the only ones populating Terra for ca. 2 billion years (37).

The first unicellular organisms (bacteria) appeared on Earth about 3.5 billion years ago. Multicellularity evolved in myxobacteria ca. 2 billion years ago, the first eukaryotes appeared 1,8-1,4 billion years ago, and animals and plants appeared 600 million-1 billion years ago (Michod, 2007). It took a long time for animals to develop as complex multicellular forms (35). The discovery of the Ediacara fauna (Australia) after the year 1950 certified the presence of metazoans on Earth ca. 650 billion years ago (6). The Cambrian Period was marked by a veritable "explosion" of life on Earth and an obvious diversification of multicellular organisms enhanced by the increased oxygen levels in the waters and in the atmosphere due to photosynthesis (Fig. 1).

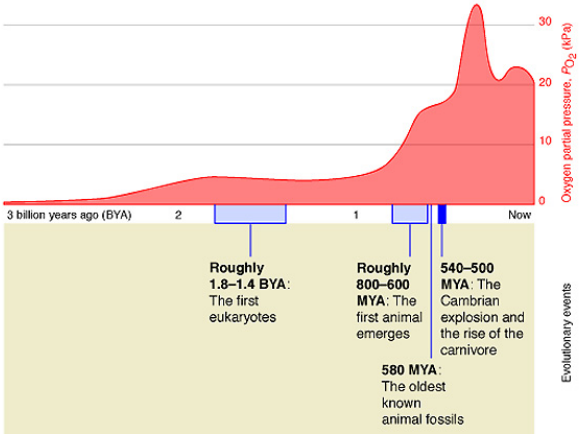

Fig. 1 - Evolution of eukaryotes and oxygen partial pressure on Terra during time (https://www.wired.com/2014/08/where-animals-come-from/)

The presumable transformation series that plants underwent in their transition from unicellular to multicellular state is: unicellular → colonial → filamentous (unbranched→ branched) → pseudoparenchymatous → parenchymatous (we define parenchymatous tissues as those in which cells can divide in all three planes of reference). The transformation series for fungi was shorter, unicellular or siphonaceous (coenocytic) fungi giving rise to multicellular fungi such as asco- and basidomycetes which consist of unbranched filaments that form a pseudoparenchymatous tissue (22). Choanoflagelates and Metazoa shared a unicellular common ancestor. However, some authors (14) consider that this common ancestor might have had an early form of multicellularity that became more robust and was lost in the Choanoflagellate lineage. The mechanism of invention of new genes and their integration to create the network of cell signalling and transcriptional regulation fundamental to metazoans, still remains a mystery.

Niklas and Newman (2013) show that the Holozoa followed the transformational series: unicellular → colonial → parenchymatous. Subsequently, evolution gave rise to functionally differentiated cell types (sponges), multilayered (placozoans), with interior cavities (chtenoforans, cnidarians) and probably those with additional layers and body cavities (mollusks), and segmented (annelids). Animal body plans are various, multi-layered, hollow or with nested cavities, elongated, segmented and appendage-bearing, and with organs with similar morphology. The authors show that even though examples of unicellular organisms descending from multicellular organisms do exist, once an organism reaches complex multicellularity and passes through the export-of-fitness phase, its capacity for evolutionary reversion to a simpler state is reduced, for it has nothing or little to do with selection on fitness grounds.

Since animals have grown in a saturated medium in microorganisms, some authors believe that through their abundance and diversity, microbes have played a major role in the appearance and evolution of animals (35), and moreover, that animals would actually be „host-microbial ecosystems”. McFall-Ngai (2013) considers that bacteria are essential partners in the digestive system of animals (from termites to humans) and that over 1/3 of human genes originated in bacteria (the hologenome theory). Some authors consider that viruses, too, have probably played a

role in the evolution of the living world, in the transition from the ARN to DNA world. Moreover, Koonin and Dolja (2013) show that *"Coevolution of viruses and host defense systems is a key aspect in the evolution of both viruses and cells, and viral genes are often recruited for cellular functions*".

In the table below we present the main differences between unicellular and multicellular organisms (28, 38):

Unicellular organisms	Multicellular organisms
The body of the organism is composed of a single cell. Body organization is simple.	The body of the organism is composed of numerous cells. Body organization is complex.
The activity(functioning) of the organism is carried out/controlled by a single cell.	Different cells in the organism are specialised to perform different functions.
Division of labour is at the organelle level. Low level of operational efficiency.	Division of labour may be at cellular level, tissue level,organ and organ system level. High degree of operational efficiency.
Usually prokaryotic in nature.	They are mostly eukaryotic in nature.
The cell has the same role for itself and for the organism.	The cells have double role: one for themselves and another for the organism.
The cell body is exposed to the environment on all sides.	Only outer cells are exposed to the environment. Inner cells perform other functions.
Any injury of the cell can cause death of the organism.	Injury or death of some cells does not affect the organism, as they can be replaced by new ones.
Small size of the cell body because of the limit imposed by surface area to volume ratio.	The organism can attain large size due to multicellularity.
Life span is usually short due to heavy load of work.	Life span is longer due to limited load of work for each cell type.
Reproduction is by vegetative/asexual methods. They reproduce quickly and in great numbers.	Reproduction is sexual type.
Few introns in genome.	Many introns in genome.
Good capacity of regeneration and power of division.	Capacity of regeneration decreases with increasing specialization. Some specialized cells lose power of division.
Cell differentiation does not exist.	Cell differentiation is evident.
Nutrition by engulfing food.	Nutrition is by specific organs or by food reproduction. They can be autotrophs or heterotrophs.
They are microscopic in nature.	They are macroscopic in nature.

The emergence of multicellularity in green algae in the order Volvocales

Some authors have raised the question of how multicellularity appeared and whether it involved a massive restructuring of the genome. To answer this question, Prochnik et al. (2010)

analysed the genome of two green algae species in Volvocales Order: *Chlamydomonas reinhardtii* - unicellular and *Volvox carteri* - multicellular. Molecular phylogenetic analyses have revealed that the volvocine species evolved from a common ancestor at least 200 million years ago (during the Triassic period) and it took this algae 35 million years to complete the transition (8). Unlike *Chlamydomonas*, *Volvox* are characterized by asymmetric cell division and embryonic morphogenesis, (13). These algae have three independent origins, if we take into account the specialization of their vegetative and reproduction functions. They are flagellated, photosynthetic, facultatively sexual, haploid eukaryotes, with varying degrees of complexity (20). As a matter of fact, the evolution over time of green algae from unicellular to multicellular state followed the steps: - ca. 223 million years ago a species of single-celled green algae began forming aggregates of cells using secreted proteins and sugars; - ca. 200 million years ago cell division in multicellular algae (the number of cells produced) began to be controlled genetically; - then the cells of these algae began to orient their flagella in the same direction so that to control the movement direction of the organism; - ca. 100 million years ago reproductive cells differentiated in some of these volvocine algae and there were developed cells with specialized functions (26).

Chlamydomonas reinhardtii is a haploid unicellular eukaryote species (about 10 μm), with 2 flagella (which enhance motion in water) and can reproduce both sexually and asexually. *Volvox carteri* is a multicellular eukaryote (ca. 350 μm diameter), with relatively low rates of cell differentiation, with only 2 cell types: - ca. 2000 - 4000 small biflagellate somatic cells that form a sphere (a coenobium) with flagella disposed at its surface (which provide coordinated swimming); - ca. 12 - 16 large reproductive cells (gonidia), reproduction is both sexual and asexual (male, female and vegetative colonies), (Fig. 2). The simplicity of their organization has made *Volvox* species a model system useful for exploring multicellularity, as they are organisms made up of a few thousand cells (instead of trillions, like in the case of other organisms) and only two cell types. It is believed that cellular differentiation into sterile somatic cells has evolved at least three times within the clade and further specialization of the reproductive cells occurred in at least two independent lineages, (9).

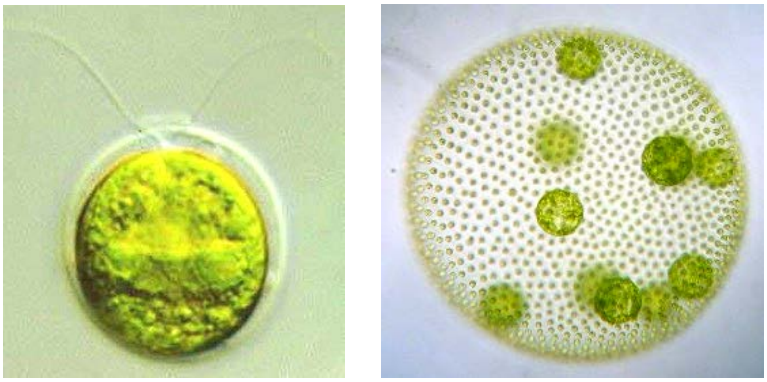

Fig. 2 - *Chlamydomonas reinhardtii* (left) și *Volvox carteri* (right)
(Prochnik et al., 2010)

Prochnik et al. (2010) sequenced the *V. carteri* genome and compared it with that of *C. reinhardtii* (sequenced in 2007) and observed that the two species have a relatively similar number

of protein-encoding genes, 14520 and 14516 gene (only 32 of the genes present at *Volvox* have no relatives in *Chlamydomonas*). The size of the genome at *Volvox* is superior (ca. 138 million base pairs, due to transposons and repetitive DNA) in comparison to *Chlamydomonas* (ca. 120 million pb). Two groups of genes have several clones in *Volvox*: the genes that determine glycoprotein synthesis (which form extracellular matrix) and the genes that encode cyclins (proteins involved in cellular division). In addition, the extracellular matrix in *Volvox* takes up over 99% within the volume of the colony and has, among other things, functions as cell orientation and sex induction (Gille et al., 1983, 1984; Hallman and Kirk 2000 - cited by Herron, 2016).

Following the comparative study on the two species, Prochnik et al. concluded that the transition from unicellular to multicellular life did not require large changes in gene content (number of genes). The transition was also enabled by mechanisms of regulation of gene expression: alternative splicing (ca. 2,9% of genes in *Volvox*), gene duplication, the intervention of cis-regulatory elements of gene expression, and of microRNAs (that post-transcriptionally regulate gene expression), (13, 30).

A mutation with major implications in animal evolution

Early last year, the results of molecular phylogenetic research of a team of American biochemists (published by Anderson et al., 2016), led by Professors K. E. Prehoda, J. W. Thornton and N. King, have aroused a lot of interest and have been widely commented in the scientific world (12, 21, 31, 32, 33, 39), being considered perhaps the most important breakthrough of the year in the field. They found that a seemingly minor genetic event (a mutation/substitution) changed the function of a cellular ancestral protein, which became essential for organizing the multicellular structures of the animal world. What is it about? Here is how Thornton describes the importance of his team's research: *"Our experiments show how biological complexity can evolve though simple, high-probability genetic paths*".... and, "*Before the last common ancestor of all animals, when only single-celled organisms existed on Earth, just one tiny change in DNA sequence caused a protein to switch from its primordial role as an enzyme to a new function that became essential to organize multicellular structures*", (12).

Some authors consider that by phylogenetic studies of relevant living organisms, by comparative investigations on animals and their close unicellular and colonial relatives, one can rebuild cell biology and reconstruct the genome of the last animal's ancestor on Earth - the hypothetical Urmetazoan. Studies of this kind support the idea that Urmetazoan possessed a layer of epithelium-like collar cells, fed on bacteria, reproduced by eggs and spermatozoa, and developed through cell division, cell differentiation and invagination (25).

The Choanoflagellates are considered to be the closest relatives of animals, (3, 5, 14, 16, 25); they are single-celled microeukaryotes or form simple colonies (of equipotent cells) and they live in marine and freshwater environments. There are over 125 known species of choanoflagellates. Cell morphology and feeding behaviour have been conserved and resemble structurally and functionally to a group of specialized cells– *sponge Choanocytes*. Some choanoflagellates may form multicellular colonies through the microvilli collar or through fine intercellular bridges (somewhat similar to the ring canals that link spermatogonia or ovogonia), (3). Fossil sponges have been found in Cambrian (over 500 million years old), organisms with several cell types: *Pinacocytes* (which form the outer layer), *porocytes* (for the pores in the body), *sclerocytes* (producing the spicules of skeletal system), *archeocytes* (with digestion functions, gamete production, tissue repair and regeneration) and *choanocytes* (which cause fluids flow

through the body). Choanocytes strikingly resemble the cells of unicellular choanoflagellates, which has led to the hypothesis that these organisms and animals are sister groups. The next level of complexity reached by animals is represented by hydrozoans, which contain more types of cells than Porifera and are quite complex as true predators (16).

Getting back to *Choanoflagellates*, one of their representatives is *Salpingoeca rosetta*, a uniflagellate organism – which can be unicellular or it can form simple colonies of either chain-like or rosette-like morphologies. Ultrastructural analyses showed that cells in rosette and chain colonies are connected by a combination of intercellular bridges, extracellular matrix, and filopodia (3). The formation of rosettes is regulated by the lipids in the bacteria on which this protozoan feeds. In fact, some authors appreciate that through their abundance and diversity, bacteria "*have exerted important signalling influences on diverse animal and non-animal lineages*" (19, 35). Ample studies in recent years on choanoflagellates, conducted by King, Dayel, Fairclough and others have shown that the rosettes of this protozoan are formed by cell division rather than by cell aggregation, which support the hypothesis that transition from unicellular to multicellular organisms was achieved by repeated cell divisions, (36).

By sequencing the genome of some choanoflagellates there have been identified common genes with multicellular animals (sponges, cnidarians, and ctenophores), such as cell adhesion genes, signalling genes and extracellular matrix genes, which suggests that these genes had developed prior to the transition to multicellular animals. This means that the two groups of organisms share a common ancestor. Genome and transcriptome of *S. rosetta "suggest that the genome of the last common ancestor of choanoflagellate and metazoans contained genes and domains that orchestrate development in modern animals but underwent important changes in gene content and regulation en route to the evolution of the first metazoan."*, (5).

Interestingly, some choanoflagellates which do not form colonies (*Monosiga brevicollis*, for instance) also have in their genome genes that in multicellular animals encode proteins involved in cell adhesion, development and differentiation, which in their case may serve in relation to the environment, (35). The fact that *M. brevicolis* exhibits specific metazoan protein domains involved in signalling and adhesion suggests that they appeared prior to the divergence of choanoflagellates and metazoans. It is presumed that the transition of metazoans from the unicellular ancestor was made through a colonial intermediate– *Urblastea*, composed of choanoflagellate type cells. (5).

Until about 1 billion years ago, the Earth was only populated by unicellular organisms. The emergence and evolution of multicellularity still remains a mystery, although steps have been taken to decipher this phenomenon. In their molecular phylogenetic research, Anderson et al. (2016) show how by duplication and divergence of an ancient guanylate kinase (gk) before the divergence of animals and choanoflagellates from unicellular organisms and the appearance of true multicellularity, a scaffolding protein developed - GK_{PID} (the guanylate kinase protein interaction domain), which function in cell adhesion and mitotic spindle orientation. This protein domain appears to be essential in tissue formation, (2).

The orientation of the mitotic spindle relative to cell axis and neighbouring cells is essential in the formation of the organized tissues. In *Drosophila*, for instance, epithelial cells divide symmetrically - perpendicular to the apical-basal axis, while the neuroblasts divide asymmetrically - parallel to this axis. In both cell types, receptor-independent G-protein signalling involving the GoLoco protein Pins has its definite role in the mitotic spindle orientation. For proper mitotic spindle orientation in neuroblasts and epithelial cells, the Pins protein associates with the Mud protein, both localized on the cellular cortex. For correct orientation of the mitotic spindle in neuroblasts and epithelial cells, the Pins protein associates with the Mud protein, both localized on

the cellular cortex. Moreover, Mud protein localizes to centrosomes during mitosis (independently of Pins) to regulate their organization.

GK_{PID} forms in the animal cell a molecular complex whose functions have been studied in *Drosophila melanogaster* neuroblasts and it plays a similar role in birds and mammals. The complex consists of the GK_{PID} of Dlg protein (Discs-large protein – a membrane associated protein with several molecular domains) which serves as support for mitotic spindle orientation by coupling two molecular partners: an "anchor" protein located inside the cell membrane (Pins - in insects or LGN - in vertebrates) - which indicates the position of the neighbouring cells, and a motor protein belonging to kinesin-3 family (Khc-73) - which binds to the microtubules of the mitotic spindle and pulls the chromosomes (monochromatic) towards the anchor, thus orienting the daughter cells resulted from division relative to their neighbouring cells, (Fig. 3).

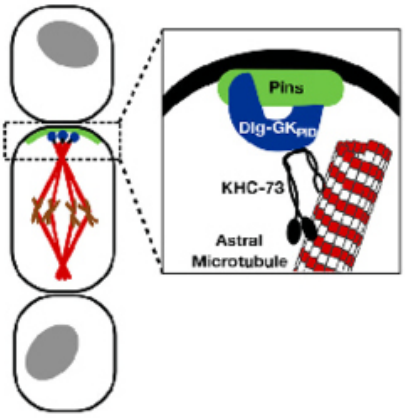

Fig. 3 - The GK_{PID} of the protein Discs-large (Dlg, blue) serves as a scaffold for spindle orientation
by physically linking the localized cortical protein Pins (green)to astral microtubules (red) via the motor protein Khc-73 (black), (Anderson et al., 2016).

Drosophila discs large protein (Dlg) is a member of the membrane-associated guanylate kinase homolog family. It serves in cytoskeleton organization, localization of membrane proteins, and apicobasal polarity of epithelial cells. The *dlg* gene is defined as a tumour suppressor gene, (Woods et al., 1996). Kinesin-3 motor protein family includes: Kinesin-73 (Khc-73) – which plays a role in the mitotic spindle polarity in *Drosophila* neuroblasts, KIF1A in mammals and Unc-104 in *Caenorhabditis elegans* (10).

The GK enzyme and the GK_{PID} protein have similar sequence and structure but they have completely different functions. GK is a univesal enzyme in the living world (it catalyses the transfer of phosphate group from ATP to GMP), whereas the GK_{PID} family proteins are only present in Filozoa (Animals, Choanoflagellates and Filasterea). Observations on the Dlg-GK_{PID} protein and on guanylate kinase suggested that these two proteins may have evolved from an ancient gk enzyme.

One of the members of the research team, Professor Thornton has pioneered technics for reconstructing ancestral genes. In order to verify the above mentioned hypothesis, Anderson et al.

(2016) have evaluated the phylogeny of the protein family- gk enzyme/GK_{PID} protein using maximum likelihood method and the sequences of 224 family members from over 40 animal species (found on various branches of the family tree) they have created their genealogy on computer and by „travelling in the molecular past” of these species, reconstructed the ancient forms of the analyzed proteins, trying to deduce their molecular parent (the sequence of the ancestral protein), (2, 34).

The authors focused on two ancestral nodes, namely Anc-$GK1_{PID}$ protein (from which Filozoa descended) and Anc-gk_{dup} (which existed before the gene duplication) and came up with the hypothesis that transition from the function as a gk enzyme to that of orienting mitotic spindle occurred during the phylogenetic interval between Anc-gk_{dup} and Anc-$GK1_{PID}$.

They synthesized the specific DNA sequences specific and transferred the resulted genes in Drosophila S2 cell cultures, ko for the gk and GK_{PID} genes. They found that Anc-gkdup protein is an active guanylate kinase enzyme and does not have the ability to bind Pins or to orient the mitotic spindle, whereas Anc-$GK1_{PID}$ does not exhibit enzymatic activity but binds Pins with moderate affinity and has high efficiency in the orientation of the mitotic spindle in cell cultures.

Then the authors also reconstructed Anc-$GK2_{PID}$, a more recent progenitor of Dlg proteins in metazoans and found that this protein has high binding affinity for Pins and of orienting the mitotic spindle. On the basis of these results, they concluded that spindle orientation function of $GK1_{PID}$ arose before the divergence of the choanoflagellates and the appearance of metazoans, being an important moment in the evolution of complexity in the animal world (1, 2).

By studying proteic sequences of Khc-73 and Pins in databases, the authors found that Khc-73 orthologues are present in Animals, Choanoflagellates and Filasterea, but not in fungi. Thus, they concluded that Khc-73 gene is as old as the Filozoan ancestor. Then they found Pins orthologues in choanoflagellates (*Salpingoeca rosetta* and *Monosiga brevicollis*) very similar in sequence and domain structure, but not in Filasterea, which indicates that Pins evolved after the emergence of GK_{PID}. They also noticed that *S. rosetta* GK_{PID} can bind the Pins protein of *Drosophila* but not its own Pins, which suggests that this association appeared after animals diverged from choanoflagellates.

Finally, Anderson et al. (2016) sought to identify the genetic mechanism by which the GK_{PID} evolved its capacity to bind Pins and they concluded that the candidate mutations occurred during the phylogenetic interval between Anc-gk_{dup} and Anc-$GK1_{PID}$. They analysed the amino acid changes occurred in two regions of these proteins and noticed only five preserved amino acids in GK_{PID} descendants. Then they tested what amino acid substitutions change its enzyme function for that of binding Pins and found that either of two substitutions (s36P or f33S), in the hinge region of the protein, could produce this change of function.

Besides the worthy contribution to deciphering some mysteries of evolution, the American team’s researches seem to have a great practical impact. One of the team members, Professor Kenneth Prehoda, believes that the information obtained could also be useful in cancer research. It is known that cancer cells cease to function as „team members” (as the author expresses it) and take the individual road of unrestricted division, with a behaviour that resembles unicellular rather than multicellular organisms. If the mitotic spindle of the cells in division is not properly aligned in relation to the surrounding cells, a tumour tissue may form instead of a normal one. Therefore, a thorough knowledge of the molecular events and processes and regulatory mechanisms in unicellular and multicellular organisms could contribute to the development of cancer research, (21).

The work published by the American researchers at the beginning of 2016 stirred up the interest of the scientific world and press, giving rise to many favourable comments but also a number of critics that should be taken into consideration. Professors Prehoda and Thornton themselves, two of the American research team leaders, participated in disseminating information, giving a lot of interviews about the result and the impact of these investigations. Some critics consider, among other things, that working with living cells makes "travelling in the molecular past" difficult, that the experiments on the positioning in Choanoflagellates require a series of clarifications, and that the authors overstate the impact of Khc73-Dlg-Pins complex in mitotic spindle orientation in all animals and the belief that this is the only novelty of animals etc, (33).

Some of these criticisms can be justified, but they should not be exaggerated and the American team's contribution to deciphering an important event in the evolutionary history of the GK_{PID} molecular complex should not be understated. Obviously, it was not this mutation alone that ensured the evolution and complexity of the animal world, as there are multiple factors and mechanisms contributing to this phenomenon: besides gene mutation and acquisition of new genes, there is also gene interaction, gene duplication, gene transposition, regulatory mechanisms of gene expression (alternative splicing, cis-regulatory factors, micro-RNA) etc.

In fact, Anderson et al. (2016) themselves admit that many aspects in this history of animal evolution still remain to be clarified, including how and when the interaction between GK_{PID} and Khc-73 evolved, the mechanisms by which Pins acquired its linker and GoLoco sequences, and the relationship of these components to other pathways and molecular complexes involved in animal spindle orientation.

CONCLUSIONS

The aim of this paper was to show how seemingly minor genetic events occurring in the genome of some organisms can have important consequences for the evolution of the living world.

Two recent studies have been used as arguments: Prochnic's study (2010) – on *Chlamydomonas* and *Volvox* green algae and Anderson's et al. study (2016) - on the molecular phylogeny of GK_{PID}, which show that the transition from unicellular to multicellular organisms did not require major genetic restructuring.

Small differences in gene content can lead to important differences between organisms. At times, point mutations were enough for some proteins in living organisms to acquire new functions.

The transition from unicellular to multicellular organisms was made possible not only the acquisition of new genes, but also by gene interaction, gene duplication (that allowed one of the gene variants to acquire new functions), regulatory mechanisms of gene expression (alternative splicing, cis-regulatory factors, micro-RNA) etc.

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