

Toward a New Understanding of Multicellularity

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Abstract

Multicellularity is a widespread development of life on Earth and so is the use of the term. The term multicellularity is currently used to characterize a broad spectrum of cellular manifestations, from unicellular symbiotic arrangements, to colonial gatherings and organisms that undergo cell specialization. These manifestations are remarkably distinct and therefore should have different designations. Here we divide multicellularity into facultative and obligate life cycles, reflecting the divergence between organisms that adopt multicellularity if environmentally triggered and organisms that are multicellular from conception and cannot complete their life cycle without undergoing a multicellular phase. We then compare multicellularity throughout the three domains of life and identify the circumstances involving the emergence of obligate multicellularity. It is here hypothesized that obligate multicellularity results from extended cooperation between different life entities adopted as a strategy to survive prolonged low-fitness periods.

Keywords: multicellular, evolution, totipotency, Eukarya, Bacteria, Archaea

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Introduction

The earliest claims of multicellular features date back to the “Great Oxygenation Event” (2.45-2.22 Gya) represented by cyanobacterial fossils [1]. The argument is that these organisms had (and its descendents still have) the ability to differentiate and compartmentalize certain metabolic functions such as reproduction and nitrogen fixation [2]. Therefore they meet the requirements for multicellularity, if solely defined by the existence of cell specialization. The wide availability of oxygen at a planetary scale assumed to be caused by the photosynthesis performed by unicellular morphotypes of cyanobacteria is thought to have propelled the emergence of these multicellular cyanobacteria. Though the potential for multicellularity could have existed long before the “Great Oxidation Event” (GOE), it deeply altered the biota on Earth and allowed for the appearance of cell compartmentalization within new evolving forms [3]. Donoghue and Antcliffe [4], however, suggested that the earliest evidence for multicellularity was independent of the GOE and revolves around 2.1 Gya old fossils of large colonial organisms found in Gabon.

Dating the earliest emergence of multicellularity may be a peripheral task in understanding it, because there is wide spread evidence that multicellularity has emerged multiple times, independently and at different times [5] and potentially through different mechanisms. Unlike other major events in the evolutionary history of life on Earth the emergence of multicellularity is not a unique and isolated event. This is indicative of some relative ease in this evolutionary step. This ease can be related to the molecular mechanisms [6] allowing the transition (such as simple mutational events); environmental (abiotic and biotic) conditions favorable (or not detrimental) to this transition occurring frequently, or a combination of these [7, 8]. The widespread existence of multicellularity is certainly related to the adaptive advantages associated with it, such as an increase in size [9, 10]. The main contention that arises between various authors is how to define multicellularity, and whether to consider multicellularity as exclusively present in Eukarya, or to extend it to the other two domains of life as well [11-16]; and what factors can foster its emergence. In this work we seek to address all these three topics: 1) re-define multicellularity and distinguish facultative multicellularity from obligate multicellularity; 2) compare archaeal and bacterial multicellularity to

eukaryotic multicellularity, according to the definitions put forward in (1); and 3) identify the circumstances surrounding the evolution of obligate multicellularity.

1. What is Multicellularity?

Most fundamentally multicellularity is a term used to define a living being constituted by more than one cell. It is used in contrast to unicellularity, which describes single-celled organisms. However the dividing line is not clear-cut, because there are many colonial organisms (single-celled beings which can physically attach or be closely connected and are interdependent with others), facultative multicellular formations (unicellular organisms that can adopt multicellular functions if environmentally triggered) and organisms that spend most of their life cycle as unicellular organisms but require a brief multicellular stage at some point in their development in order to reproduce. King [17] defined a multicellular organism as one “possessing stably adherent cells whose activities are coordinated or integrated”. This is a loose definition as the concept of stable adhesion can be flexible enough to include colonial organisms under the multicellular grouping. Bell and Mooers [18] define multicellular organisms as “clones of cells that express different phenotypes despite having the same genotype”. This definition, as simple as it is, captures in our view the most distinctive feature of multicellularity. It is indeed differentiation and eventually specialization within a group of identical cells that can account for the rise in complexity provided by differential expression of the same genome. One clarification might be added here and that is that this differentiation is cooperative and not competitive; required for the survival of the organism; genetically predetermined; non-reversible; and transmitted to progeny. Smith [19] has exposed the distinction between cooperative and competitive differentiation (although in another context) with the fitness of each type of cell inherently dependent and qualitatively identical (if not quantitatively) on the fitness of another. The idea that biological complexity can be classified according to the division of labor (e.g., number of cell type specializations) has been developed by Limoges [20], and more recently by Bonner [21].

Multicellularity, in its facultative form is quite common, indicating that it is a rather successful organismic strategy. Its obligate form is also common. Multicellularity has transformed life on Earth and we need to comprehend why it evolved and what the evolutionary trade-offs associated with its development are. Cooperation can be highly

beneficial but it includes inherent risks. Cooperation emerges out of a need to overcome an obstacle difficult or impossible to be dealt with individually (e.g. by a single cell). Cooperating parties will initiate and maintain that cooperation only for as long as needed and will take advantage of the other party if given the opportunity (e.g., development of parasitism). Unicellular organisms which engage in close associations face high survivability risks due to the possibility of failed cooperation, but in case that association is successful, their fitness will substantially increase. Obligate multicellular organisms have taken that risk with full commitment. Table 1 summarizes distinctive characteristics of facultative and obligate multicellular organisms.

Obligate multicellular cells lose totipotency, and a failure in cooperation will result in the demise of that organism. While a unicellular cell or even a cell within a facultative multicellular organism can be qualified as a minimal unit of life, a cell within an obligate multicellular organism cannot. A cell of an obligate multicellular organism cannot survive nor reproduce on its own (even undifferentiated cells of a multicellular organism, which occur as embryonic cells or cancer cells need to be nourished in a multicellular environment). Obligate cells within a multicellular organism have no choice but to cooperate in order to survive.

2. Evidence for an Evolutionary Path to Multicellularity

2.1. Bacteria

Bacterial multicellularity has been claimed for various phylogenetic groups including *Cyanobacteria*, *Actinobacteria* and *Proteobacteria* such as *Myxobacteria* (e.g., [1]). The issue of bacterial multicellularity will be discussed here on an intriguing example of the myxobacteria (though it exists in some other phyla as well).

Myxobacteria are Gram-negative (δ -*Proteobacteria*) unicellular rod shaped bacteria that are conspicuously present in soils worldwide. Their lipopolysaccharide membrane is similar to that of most bacteria. Myxobacteria are strictly aerobic organotrophic and mesophilic organisms [22]. These microorganisms are able to form mobile, predatory communities known as “swarms” that feed cooperatively. Under starvation, the cells initiate a developmental program that results in the formation of fruiting bodies filled with myxospores, which are similar to other bacterial spores. This lifestyle requires the cells to be environmentally sensitive and

Table 1 – Comparison of multicellular properties between facultative and obligate multicellular organisms. This can be used as a classification tool of obligate multicellularity. If a positive answer to all the parameters is found then the organism should be classified as an obligate multicellular organism (modified from [51]).

Multicellular Property	Facultative Multicellularity	Obligate Multicellularity
Clones of cells with same genotype but different phenotype	Yes	Yes
Cooperative cell differentiation	Yes	Yes
Cell Differentiation required for survival of organism	Environmentally determined	Yes
Cell differentiation genetically predetermined	No, occurrence and timing of cell differentiation is genetically controlled but environmentally determined	Yes
Cell differentiation is non-reversible (at the individual level)	No, early reversal of differentiation can occur with no consequence to the fitness of the organism, or with a positive effect on it	Yes, reversal causes reduced fitness or death
Cell differentiation transmitted to progeny	No, manifestation of multicellular functions by the parental strain does not increase <i>per se</i> the probability that the offspring will develop them	Yes

able to communicate with each other to coordinate movement and behavior [23].

The formation of fruiting bodies can be described as a cooperative morphogenesis by the vegetative swarming cells. A large number of swarming cells form aggregates and lose their individual behavior (due to molecules formed on the cell surface that cause cells to stick together). This results in an unstructured agglutination of masses of cells that autolyse to about 65-90%. This agglutination is followed by the formation of the fruiting body structural elements (stem, base plate, sporangium wall). The complete process of fruiting body formation takes 12-14h under optimal conditions. Environmental factors, extracellular signal molecules and pheromones play a role in determining the developmental cycle. The involved molecules are low molecular weight lipophilic compounds that are excreted upon nutrient deficiency and considerably enhanced by light. These pheromones induce fruiting body formation. Myxospores allow the survival of myxobacteria during periods of unfavorable living conditions such as cold and heat periods, dryness,

acid pH or anoxic conditions [24]. The fruiting bodies are developed to start a new life cycle with a large population: to hydrolyze extracellular biopolymers together in a swarm using common exoenzymes and thus utilize nutritional sources with maximal efficiency. Myxobacteria present a striking case on the path to obligate multicellularity. But this type of multicellularity is not strictly necessary for their continued existence under favorable conditions; neither do they enter this state without an environmental trigger, which takes the form of unfavorable physical parameters and starvation. Once the multicellular development is initiated, it is remarkably fast in execution (in a few hours an amorphous agglomerate of cells transforms into a fruiting body with all its well-defined different structures) indicating that myxobacteria have acquired a multicellular developmental plan. The use of this type of multicellularity in the form of fruiting bodies in myxobacteria is a means to achieve a maximal propagation of myxospores. Multicellularity in myxobacteria is therefore a phase that is transitory, and not obligatory. This is a distinct difference to

obligate multicellularity, where organisms have no “choice” but to be multicellular.

2.2. Archaea

There have been some reports of structures adherent to surfaces as well as floating non-adherent structures (e.g., [25]), interpreted by some to represent multicellularity. Most of these interpretations refer to an association of Archaea with bacteria. It is well known that under anoxic conditions in marine sediments anaerobic methane-oxidizing Archaea can form synergistic structured consortia with sulfate-reducing bacteria [26]. Reports of associations between these two domains of life are not uncommon, but they do not represent a multicellular organism. Classifying these associations as a multicellular phenomenon would imply that any symbiotic interaction would have to be similarly classified. Some other Archaea can form heterogeneous biofilms. *Archaeoglobus fulgidus* is an anaerobic marine hyperthermophile that can secrete a biofilm under environmental stress. The secreted biofilm consists of proteins, polysaccharides and metals, performing both the function of a protective barrier and a reserve nutrient [27], similarly to biofilms observed from *Methanococci* and *Methanobacteria*. This may indicate that biofilm formation is a common stress response exhibited by Archaea. No cell specialization is present, however, thus no multicellular organism. The massive production of a protective and nutritious layer is noteworthy but it is simply the result of cooperation among individuals of the same species. The same reasoning applies to *Methanosarcinae*, which is able to form lamina, a flat sheet of connected individual cells of different sizes and shapes, some of them actively dividing [28].

2.3. Multicellularity in Eukaryotes

There are several eukaryotic branches widely accepted to hold multicellular life forms [17] including Opisthokonts, Excavates, Amoebozoa, Plants, Alveolates, Heterokonts, and Discicristates, indicating that multicellularity appears to have emerged not only once, but instead multiple times. We will focus in the following discussion on the Opisthokonts.

Opisthokonts are comprised of animals (Metazoa, all multicellular), Fungi (uni/multicellular), Microsporidia (a fungi unicellular sister group), choanoflagellates (Choanoflagellata, uni/multicellular), and several unicellular groups: nucleariids (Nucleariidae), ichthyosporeans (Ichthyosporae),

Capsaspora, Corallochytrium and Ministeria [29]. The location of the Eukarya root is uncertain, but King [17] places it somewhere between the Opisthokonts and Excavates. The groups most relevant in our discussion of multicellularity within the Opisthokonts are fungi and choanoflagellates, given that they both have uni- and multicellular members.

Within the fungi it is worthwhile to focus on closely related organisms that manifest different cellularities. The family Saccharomycetaceae is a model case [30]. It contains unicellular (e.g. budding yeast *Saccharomyces cerevisiae*, widely used in baking and brewing), multicellular (e.g. filamentous *Ashbya gossypii*, cotton pathogen) and even organisms which switch cellularity according to environmental cues (e.g. *Candida albicans*, causative agent of oral and genital infections in humans). Despite a high gene homology (more than 90% between *A.gossypii* and *S.cerevisiae*), their transcription and regulation machinery is very distinct. Yeast and hyphal forms have re-used conserved modules in different contexts [31]. *C.albicans* is undoubtedly the organism with the most intricate life cycle of the three under discussion. This organism is capable of rapidly and reversibly switching between yeast (unicellular) and filamentous (multicellular) morphologies [32]. The switching is environmentally triggered. To add even more to its complexity, *C. albicans* can also grow as a pseudohypha [33]. This morphology is characterized by chains of elongated cells that resemble hyphae but that are made up of yeast-like cells. Further, different strains have different switching repertoires. The fact that these three species are so closely related and yet display strikingly deep divergences in such fundamental biological features such as ploidy and cellularity should be interpreted as a sign that what most of us usually consider a gigantic evolutionary step may be quite simple to achieve functionally. There is much to be learned about fundamental biology from fungi. *S.cerevisiae* is undoubtedly a unicellular organism. *A.gossypii* is an obligate multicellular organism, as the organism can only survive and reproduce by undergoing a filamentous phase. *C.albicans*, despite all the immense complexity that this organism manifests, is not an obligate multicellular organism, but comparable to many of the bacterial examples of evolutionary steps toward multicellularity (e.g. *Cyanobacteria* and *Myxococcus*). One other group of interest within the group of Opisthokonts is the Choanoflagellates. This group is prevalently composed of unicellular organisms with some colonial forms. More than 125 choanoflagellate species have been described, all with a unicellular life-history stage [34]. No multicellular

choanoflagellates have been reported to date. However, Choanoflagellates are still relevant to the discussion here, because they are the closest known relatives of metazoans [34]. All metazoans are multicellular and the search for the last unicellular metazoan ancestor is avidly searched. Choanoflagellates are a diverse group of aquatic bacterial predators and despite being unicellular they have several signaling proteins involved in cell adhesion and signaling protein domains that were previously thought to be only present in metazoans [35]. The presence of these proteins, such as cadherin-like and C type lectin-like in choanoflagellates is somewhat puzzling given their strong role in multicellularity in metazoans. The transition in functionality of these molecules in these two groups remains unknown. Even more intriguing is the presence of otherwise metazoan-specific p53, Myc and Sox/TCF transcription factor families [34]. These are fundamental and complex cell cycle regulators with critical roles in differential gene expression. This is indicative of the presence of highly complex regulators in these organisms. Choanoflagellates are still poorly understood and it is unclear what the role of these molecules is in these organisms. At this time it appears that choanoflagellates have no multicellular organisms among its members. Therefore, fungi are the only transitional group within Opisthokonts, of which only animals are a fully multicellular clade.

2.4. Are Ciliates an Example of a different Type of Multicellularity?

Alveolates (Superphylum) are protozoa that have in common cortical alveoli (a continuous layer of flattened vesicles that support the cell membrane). Alveolates possess proteins (SFA, Striated Fiber Assemblin, which occur within the flagellar apparatus) common among protists but absent from multicellular organisms (metazoans and plants) suggesting that these proteins have been lost through evolution from the ancestral eukaryotic genome [36, 37]. The fact that these organisms have not lost their SFAs may be indicative of a predominantly unicellular life history. There are three main phyla in this division: Apicomplexa, Dinoflagellates and Ciliates. The first two phyla have no multicellular members. However the Ciliates, which are a major component of marine environments [38, 39] are a more ancient branch, and have been indicated as having some rare multicellular forms.

Ciliates have a very rare cell organization, because they have two nuclei: a small diploid micronucleus (with sexual reproduction functions)

and a large polyploid macronucleus (with asexual reproduction and cell regulation functions). The macronucleus is formed by genome amplification and editing of the micronucleus. The exact mechanisms that allow the coordination and functioning of the two nuclei are not well understood, and the process that leads to the amitotic segregation of the chromosomes in the macronucleus is unknown. But the fact that these organisms have somehow the ability to split the coordination of cell functioning without splitting the cell is remarkable. It appears that the macronucleus is secondary to the micronucleus given that it is formed from it. In essence, these cells have two interdependent nuclei, where the micronucleus needs the macronucleus in order for the cell to function properly, and the macronucleus needs the micronucleus in order to replicate. An analogy with the multicellular world would be that the micronucleus functions as a germ cell and the macronucleus as a somatic cell. Thus these two nuclei are not in different cells but function in principle, as if they were.

One might envision a scenario in which the macronucleus could asexually reproduce and retain the connection to the daughter cells. If functionality could be achieved in this arrangement, it appears that the nuclear functions for multicellularity are already in place. In other words, this division brings to mind the possibility that multicellular functions emerged from within a cell first, and then may have extended to daughter cells and not by an accidental joining of cells that further developed into the splitting of cellular functions. These organisms show that it is possible to evolve nuclear cellular task divisions without the physical separation of cell boundaries. This raises an intriguing question: Is multicellularity about distinct but cloned cells or is it about DNA copies? If it is about the cooperation of DNA copies then these organisms should be considered functionally obligate multicellular organisms, even if within one cell.

3. How did obligate Multicellular Organisms Emerge?

The shift in focus from facultative multicellularity to obligate multicellularity within organisms is not a semantic shift. It is a conceptual shift. It assumes that the unit of task division is within the genetic material and only secondarily does it involve cell boundaries. The ciliates are a living proof that task division is possible even within only one cell. What about the emergence of obligate multicellular organisms in other lineages? Did the split in entity occur first by an incomplete cellular cell division, or were the task

division mechanisms already in place by the time the physical split occurred? We seem to observe examples of both cases. There are organisms in which incomplete cellular division causes two or more cells to coexist, physically linked. In some cases the cells maintain their totipotency; in others they adopt different tasks. In this case the physical linkage between the cells is only between mother and daughter cell(s). Collaboration between these cells is evolutionarily favored given their close relatedness. This is also an example of obligatory collaboration given that the cells are physically linked (by error) and can only survive by at least not directly competing with each other. The more they cooperate, the more likely they are to survive their accidental linkage. This can lead to a division of tasks and result in obligate multicellular organisms if that mutation (that caused the physical linkage) is transmitted to the progeny and therefore perpetuated. Organisms developmentally locked into cooperation with clones are less likely to escape or deny cooperation as it most likely will result in a detrimental outcome.

A different avenue to obligate multicellularity could be through the path demonstrated by colonial organisms. This is a very different pathway from the one just described. In this case unicellular organisms that are usually closely related (but not necessarily parent-offspring like in the previous example) associate (often by gelatinous strands) when faced with particular environmental circumstances (commonly starvation and stress). These cells can maintain their totipotency or develop cell differentiation. These types of aggregations are clearly an attempt to overcome life-threatening circumstances that could not be overcome individually. Even if no differentiation takes place, the agglomeration of individual cells provides both protection (much in the same way as sought by schools of fish) and increased chances to locate food sources. These aggregations can in most cases disaggregate once the threatening conditions cease to exist. In some cases these aggregations develop into something more than a simple conglomeration, as is the case in fruiting body structures, where cells adopt a specialized function within the colony, which - by the definition put forward in this paper - becomes a facultative multicellular organism. The only characteristic that distinguishes these organisms from obligate multicellular organisms is the fact that they only develop this phenotype if environmentally triggered. In other words, this phenotype is not genetically programmed to occur at a certain time in their development, neither is it necessary to occur in order to complete their life cycle. These organisms show a plastic or facultative multicellularity, which depends on the necessity to overcome environmental

obstacles. Evolutionarily, there does not seem to be any pressure on facultative multicellular organisms to commit to further cooperation, which may lead to obligate multicellularity. Cells can aggregate when needed and if the cooperation is no longer serving individual interests, they can disassemble. The need to cooperate at all costs does not seem to be necessarily present in these arrangements. Certainly not in the same manner as between parent-offspring cells physically linked and with no choice but to cooperate.

The third possibility – genetic split – is intriguing. Ciliates are an ancient branch in a unicellular lineage (Alveolates). Their peculiar cellular arrangement is not widespread (at least nowadays). A possible reason could be linked to the lack on an increase in size, which is present when two or more cells function as a unit. It is widely claimed that the main advantage of multicellular organisms over unicellular organisms is the increase in size which allows them to feed on smaller organisms and avoid being eaten by others, along with the ability to more easily manipulate their environment, therefore gaining advantage when competing with smaller organisms [9.10]. If this is the main reason why multicellularity is widespread, then Ciliates, despite their complex molecular machinery did not achieve the size advantage. They also do not have the ability for cellular specialization given that despite their ability to split functions, there is only one cell whose functions are primarily controlled by the macronucleus. If cell boundaries between the two nuclei were to develop then we could potentially observe the emergence of a new multicellular organism. On the other hand, given the interdependency of both nuclei, a cell boundary could easily disrupt the communication and cause a total collapse of the organism.

When considering the three main avenues that are observable today and which could in theory lead to the development of obligate multicellular organisms, the most evolutionarily reasonable mechanism seems to us to be the first – an incomplete cell division. The cooperation would first be undifferentiated and then could have evolved into a differentiated cooperation that became developmental (transmitted to the progeny). The differentiation and complementarity of cell functions can have occurred first as a means to avoid the redundancy of cellular functions and energy waste associated with it. The cell would have developed the ability to move specific enzymes where they are needed and remove them from certain locations in order to stop an enzymatic function no longer needed [40] avoiding a loss of energy. By simply moving the enzymes, cell cycles could be disrupted or initiated when needed. One can envision

Putative Avenues to Obligate Multicellular Organisms

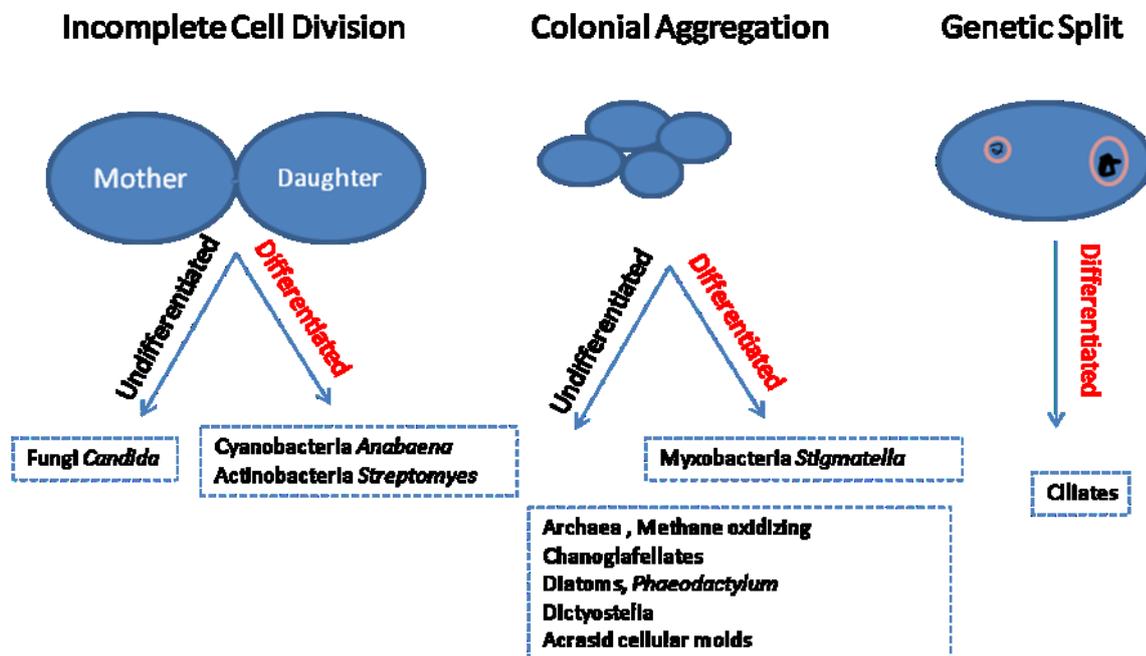


Figure 1 – Different avenues that could lead to the evolution of obligate multicellular organisms. The organisms indicated are only examples of organisms that show the indicated type of evolution. This list is not intended to be exhaustive neither all inclusive. The groups of organisms only indicate that there are examples of these types of evolutionary transitions (modified from [51]).

this happening between two cells which are accidentally connected. By focusing certain functions in one cell and others in another, redundancy is avoided and specialization with high levels of efficiency becomes possible. It would be important however that each cell holds a necessary metabolic function to the survival of the other types of cells. Otherwise, the unnecessary cell lineage could be easily discarded by the other cell. This is consistent with what we observe in obligate multicellular organisms today. In many cell lines, compartmentalization of basic metabolic processes is a precursor to the commitment of the cells to differentiate [41, 42]. A transmittal of the hypothesized ability of accidentally joined cells' progeny is more challenging, however.

The different paths to obligate multicellularity as discussed above are shown in Figure 2. Though it is unclear which path evolution chose during the natural history of life on Earth (or could have it chosen multiple routes?), the mechanistic details behind the emergence of obligate multicellular organisms appear to be relatively easy to come into existence, given the multiple independent events that have resulted in their formation. Within the same genus we find

unicellular, colonial and obligate multicellular organisms in varied combinations of ancestral state.

The transition from single-cellular organisms to a multicellular organism is one of the most fascinating open questions on biology today (Fig. 1). Given the evolutionary constraints mentioned above, the accidental incompleteness of cell division seems to us more likely to result in obligate multicellular organisms than an evolutionary pathway via colonial organisms (e.g., as suggested by [4]). Our reasoning can be summarized as follows:

- The cells are forced to cooperate. There is no easy escape from the physical linkage even if a particular cell is exposed to parasitism by the other. The development of cooperation - even at the undifferentiated state - is favored by natural selection. By contrast, colonial organisms can disaggregate relatively easily if aggregation is not favorable to the individual;

- The cells are clonal in the case of asexual beings and as closely related as possible in sexual organisms (parent-offspring). Cooperation and even cellular sacrifice (in the case of defection) are selected for because of the identical genetic material of the two or more individuals as long as at least one cell is favored

by that sacrifice. By contrast, colonial organisms are not necessarily clonal or direct relatives, which lower the advantage of communal cellular sacrifice;

- Cell specialization can occur as a result of the need to cooperate and the fact that the cellular machinery and membranes are already potentially shared and therefore can establish functional communication between the different nuclei. Cell specialization seems a lot harder to achieve among colonial formations. Despite the many examples of colonial differentiated aggregations that we can observe today none of them seem to have any evolutionary need to evolve into an obligate multicellular

We thus propose that obligate multicellular organisms were likely forced into a locked state of multicellularity, a state that facultative multicellular organisms are not subject to. This scenario is analogous to the locked sexuality exhibited by many organisms. Certainly having the choice between asexual and sexual reproduction appears a better strategy. Nevertheless, organisms with a locked state are widespread both in numbers and spatially, thus have to have some kind of selective advantage. Certainly it cannot be said that obligate multicellular organisms or sexual organisms dominate our biological world, but they are well adapted and successful organisms. If obligate multicellular organisms emerged from accidents in cellular divisions (which are quite common), then the focus in understanding complex multicellular organisms should be in those living entities that show this evolutionary pathway.

Discussion

Complex features emerge out of a group of rare fortuitous mutational events. As with all mutations, they tend to occur in larger numbers when the organism is under stress which causes an accelerated metabolism that increases the chance of errors during the replication, transduction, translation, and replication mechanisms. In a stressed and declining population, advantageous mutations will spread rapidly.

The most serious and common cause of struggle to life is the lack of energy sources (starvation) to sustain its survival and reproduction. Any mutation that causes a more efficient use of energy [43, 44] under these conditions will be advantageous to the individual and to the population at large. Only a very low fraction of mutations are advantageous, but this one critical mutation could completely change the evolutionary trajectory of life.

Cooperation is critical in this respect and the main advantage of cooperation is precisely the economy of energy at the individual level. But if we zoom out, we can also realize that cooperation eliminates the waste of energy caused by unnecessary competition among identical organisms. Cooperation is a much underestimated powerful force of life. The logical flow of energies will cause organisms, which can utilize the steepest gradients in energy [45] towards entropy [46, 47] become the best competitors. But whereas competition causes the survival of the fittest (or fit enough) and the decrease of the weakest, cooperation allows for the fittest to become even stronger and the weaker organism to survive and potentially fuse with the fittest.

Stressing factors, most common being starvation, increase the mutation rate, while at the same time favoring innovations that allow for joined efforts. It is under stressing conditions that individual barriers are most likely to fall, giving rise to new forms of cooperation. It is under this scenario that multicellularity is most likely to have emerged, perhaps during an accidental fusion event as suggested above. It seems peculiar that obligate multicellularity was not achieved within the other two domains of life (Archaea and Bacteria). Possibly, only eukaryotes were far enough evolved in cellular specialization that this path to multicellularity was feasible.

Environmental stress certainly accelerates evolution and may have been a driver of the "invention" of obligate multicellularity as well. A seemingly appropriate example is brown algae, simple multicellular organisms within the predominantly unicellular Heterokonts. Most brown algae (Phaeophyceae) inhabit intertidal zones [48] and are not closely related to the unicellular red and green algae [49]. Intertidal zones are notorious for the constant and intense abiotic and biotic stress, due to the tidal changes and dense biota, respectively. The constant exposure to different physical and chemical parameters that accompany the tidal changes such as salinity, exposure to desiccation, temperature swings, mechanical stress and the mobile biota that accompanies tides, has forced these organisms to be highly adaptable to changing conditions within a short period of time. Brown algae are clearly multicellular, and have receptor kinases that are related molecules (although independently evolved) to those linked with the emergence of multicellularity in both animals and green plants [48]. *Ectocarpus siliculosus* (brown algae) has been extensively studied and is currently used as a model organism for multicellular organisms given its simple composition. This alga has a filamentous thallus with two types of cells (elongated and round cells) from which

branches differentiate [50]. The elongated cells are present in the apices, where active growth takes place. As the organism grows and the elongated cells are distanced from the apices, they progressively differentiate into round cells. These differentiations are known to be determined by concentrations of IAA (indole-3-acetic acid, an auxin – plant growth hormone), which are higher at the apices and progressively decrease with distance to the source [50].

While the multicellular composition of *Ectocarpus* is relatively simple, its life cycle is quite complex, involving a sexual and asexual cycle as well as multicellular haploid and diploid forms. Despite the multi-pathways that this organism can follow in order to reproduce, none of them excludes the existence of a multicellular form. This undoubtedly classifies this organism as an obligate multicellular being. Thus, it is certainly not a coincidence that from all the other algae and proposed multicellular heterokonts (e.g., see [17]) only brown algae have developed obligate multicellularity.

8. Conclusions

Unicellular organisms have full control and capability to accomplish all life processes necessary for survival and replication. In facultative multicellular organisms, multiple cell arrangements can be adopted if environmentally triggered, but they are not required for the completion of the life cycle or reproduction of the organism. In obligate multicellular organisms, multiple cell arrangements are necessary for the completion of the life cycle and reproduction, and these characteristics are transmitted to the progeny. The latter type of arrangement (obligate multicellularity) is exclusive to the domain Eukarya. Here we pointed out three possible pathways to obligate multicellularity and made the case why we consider the accidental joining of two cells as the most promising route to obligate multicellularity. As demonstrated by facultative multicellular organisms, environmental stress triggers multicellular displays. We hypothesize that environmental stress also promoted even further-reaching cooperation with the eventual result of obligate multicellularity - with the evolutionary compromise that totipotency is lost for these types of organisms.

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