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REVIEW ARTICLE

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The calculative nature of microbial biofilms and bioaggregates

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Abstract Biological proliferation is optimized at various levels of organization, including the molecule (e.g. nucleic acids, prions), the cell (e.g. prokaryotic cells, eukaryotic cells), and the community (e.g. microbial biofilms, bioaggregates). Although it was initially assumed that this occurred through the genesis of information within DNA alone, it now appears that innovative design originates at other levels of organization in addition to DNA. For example, the recombination of community structures affects the proliferation rate of genetic structures; and the recombination of genetic structures affects the proliferation rate of community structures. This feedback mechanism computes compromises between the form and function of both community and nucleic acid. A nested series of proliferating objects (e.g. genetic structure, cell structure, community structure) is thus capable of continually updating the form of each object in the series. This accounts for the calculative nature of prokaryotic cells, eukaryotic cells, biofilms, bioaggregates, microbial consortia, and most other complex adaptive systems.

Keywords Biofilms · Bioaggregates · Levels of organization · Community structure · Biological consortia

Introduction

The structure and function of microbial biofilms, bioaggregates, and consortia are optimized for proliferation at many levels, including the molecule, cell, organism, community, and ecosystem. However, based on what is often referred to as the central dogma of molecular biology [18, 19], the design for all biological forms (molecule, cell, organism, community, ecosystem, etc.) originates through genetic recombination. If so, then innovation travels in one direction. The novel information is formed within DNA molecules, which then form RNA molecules, the RNA then forms proteins, the proteins form cells, the cells form organisms, the organisms form communities, and the communities form ecosystems. Thus biological innovation originates in DNA (through genetic recombination and/or mutation) and flows through a closed pipeline, with no appreciable exchange of information (communication) between the interior of the pipeline and the environment, as shown in Fig. 1. This presumes that DNA structures are innovative and able to express themselves, while other biological structures are not.

An alternative option, which more readily explains the optimization of biological design at multiple levels of organization, is that innovative information flows in two directions. It moves from DNA to community and from community to DNA; and it originates at many levels of structural organization (DNA, RNA, protein, cell, community, ecosystem). There are two such theories of bidirectional information flow: the Commoner cell theory and nested proliferation theory. The Commoner cell theory postulates that information flows bidirectionally between DNA and the cell [11, 12, 13, 14, 15, 16]). As stated by Commoner: ''DNA is a mechanism created by the cell to store information produced by the cell'' [17]. Nested proliferation theory postulates that information flows bidirectionally between DNA and the community and also flows between all levels of geometric nesting [2, 3, 8, 9]. Postulating the bidirectional flow of innovation results in the open pipeline illustrated at the bottom of Fig. 1. Note that the closed pipeline has only one port between the pipeline and its larger environment (the DNA recombination port). However, the open pipeline has several ports connecting it with the larger environment (RNA, cell, and community ports, multiple DNA

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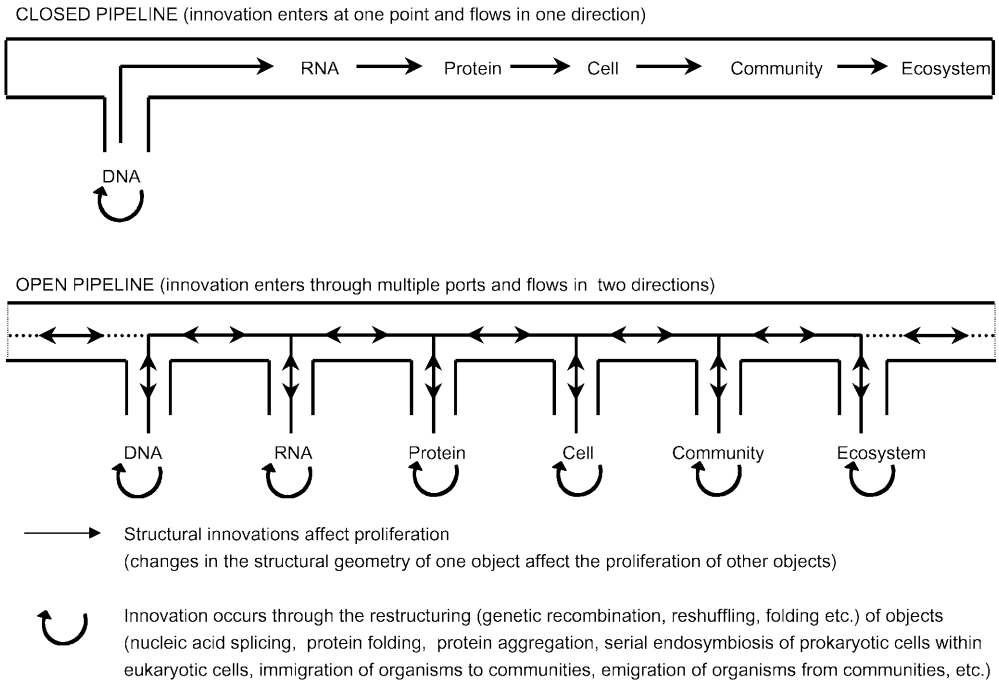

Fig. 1. The pipeline of biological innovation

and protein ports). Each port can be thought of as an environmental sensor or connection, each allowing innovative experiments on a different scale of organization (for nested objects) or from a different perspective (for neighboring objects). Within the open pipeline, innovation can flow in two directions and can enter or leave at many different locations. Innovation is lost and/or produced through the restructuring (recombination) of nucleic acids, proteins, cells, and communities via nucleic acid splicing, protein folding, protein aggregation, serial endosymbiosis, immigration, and emigration. To serve either as a source of information (communities being prominent as sources of innovation), or as a sink for information (DNA being prominent as both a source and a sink), a structure must be capable of proliferating independently or as part of another structure and it must be capable of undergoing a spontaneous change in its structural geometry (restructuring via mutation, recombination, folding, aggregation, fragmentation, etc.).

The calculative geometry of nested microbial structures

The calculative geometry of nested microbial structures is such that the proliferation of nucleic acids, proteins, cells, communities, and ecosystems continually recalculates and updates their form and function [3]. It is the nesting of proliferating structures that provides the calculative mechanism by which life evolves through bidirectional information flow. For example, DNA molecules proliferate within cells, cells proliferate within organisms, and organisms proliferate within communities. Each is both a habitat and an inhabitant. One of many calculative elements within this hierarchy is the feedback between genetic structure and community structure. Changes in genetic structure affect the proliferation rate of community structures and changes in community structure affect the proliferation rate of genetic structures. This conversational feedback mechanism computes compromises between the form and function of both community and nucleic acid. However, this is only one element in a series of calculative elements. The series is capable of calculating compromises between many objects, including DNA molecules, RNA molecules, proteins, cells, communities, etc. The form, function, and proliferation rate of each is continually recalculated and updated through a series of geometric feedbacks (Fig. 2). This requires a series of structures, each of which is capable of proliferating and of undergoing spontaneous changes in its structural geometry. It includes changes in DNA sequences (DNA sequence reshuffling via genetic recombination), changes in DNA methylation (through heritable methylation patterns at GATC sites, due to DNA adenine methylase), in RNA structure (messenger RNA sequence reshuffling via spliceosomes), in the tertiary structure of proteins (through protein folding, as in the case of prions and in the case of internal ionic and disulfide bonding), in the quaternary structure of proteins (through protein aggregation via ionic and disulfide bonding), in the structure of cells (through the serial endosymbiosis of prokaryotic cells in eukaryotic cells), in the structure of communities (through cell emigration and immigration), and through numerous other potential mechanisms. Note that there are 21 individual calculative mechanisms that could be individually extracted from this diagram (examples are shown in Fig. 3). The addition of a structural element to the series increases the number of least calculative mechanisms by the number of structural elements already present.

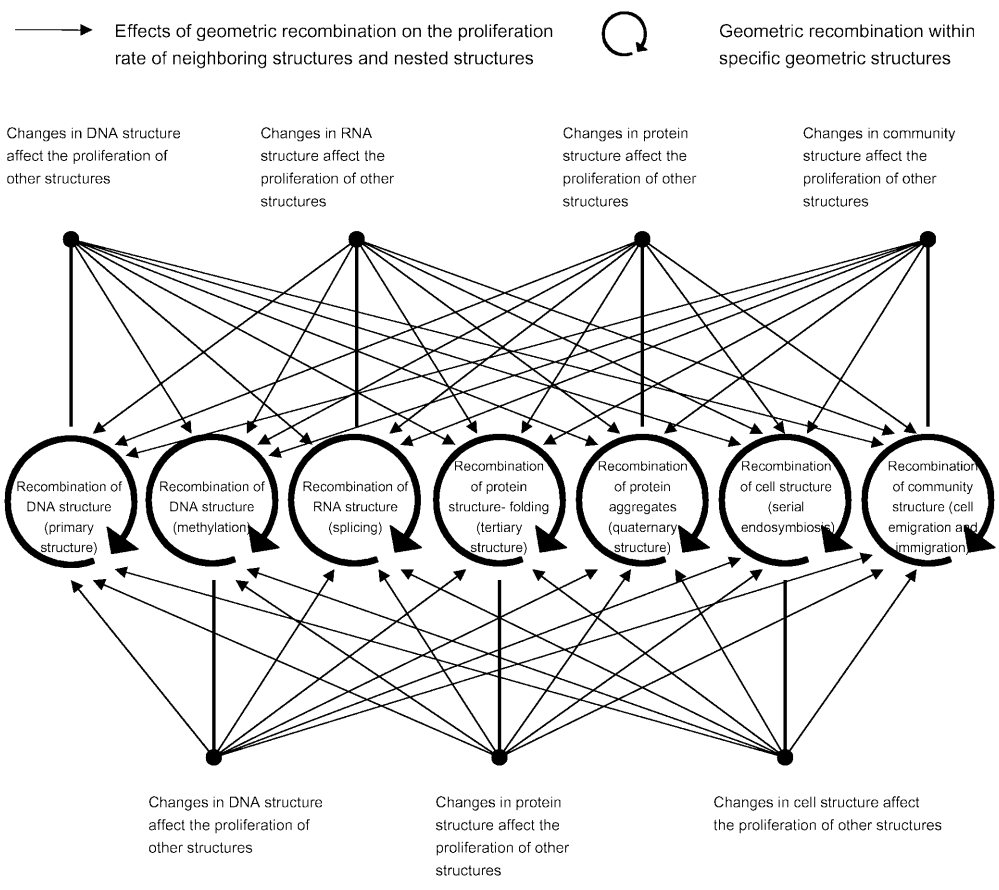

Fig. 2. Calculative geometric feedback mechanisms active in biofilm communities

Thus, it is the proliferation of nested objects that continually recalculates their form and function through the effect of their proliferation on the proliferation of other objects, most of which reside at other levels of nesting. To be active in evolutionary calculations, an object must be capable of spontaneously restructuring (either independently or through the spontaneous restructuring of a surrogate) and capable of proliferating (either independently or as part of a larger system). Thus, the emergence of life forms results from the bi-directional movement of information both vertically (between nested objects) and horizontally (between neighboring objects) among and within objects that are physical, but that are at the same time calculative (if their geometric position is strategic with regard to proliferation).

Mechanisms for restructuring DNA

As shown in Fig. 2, there are various mechanisms for the geometric restructuring of DNA, RNA, protein, cells, and communities. Each is somewhat unique. The primary sequencing of the DNA molecule in prokaryotes is restructured through conjugation, transduction, and transformation. These are represented by a single DNA port, although each is unique and might be represented individually. A separate port is shown for the action of DNA adenine methylase, which produces a heritable pattern of methylation at GATC-specific sequences by creating 6-methyladenine [27]. These methylation patterns affect gene expression and result in bacterial phase variations that regulate the production of adhesins and other gene products [24]. Thus, they represent a distinct recombinational element (i.e. a unique portal in the pipeline of innovation).

Mechanisms for restructuring RNA

RNA is normally restructured through the genetic recombination of the DNA blueprint that encodes it. However, messenger RNA also recombines through the action of spliceosomes that assemble at specific sites along the messenger RNA molecule, where they produce RNA fragments that are subsequently spliced together in new combinations to form novel m-RNA molecules expressed by the cell as proteins, although these proteins are not directly encoded as genetic sequences [10]. This allows innovation to enter the pipeline via both RNA and DNA, as illustrated in Figs. 1 and 2.

Mechanisms for restructuring protein

As in the case of nucleic acids, there are numerous mechanisms of recombination in protein. Protein

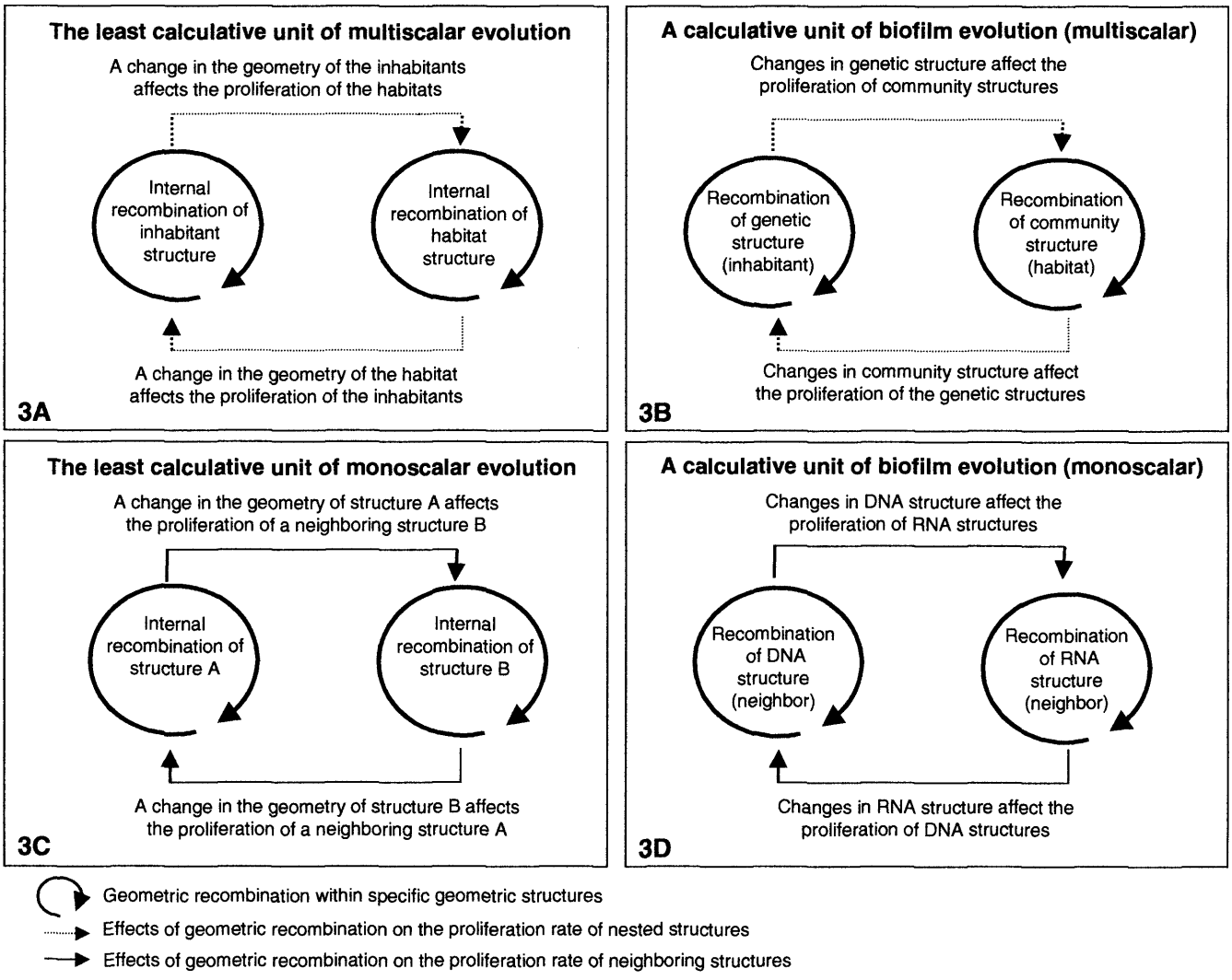

Fig. 3a–d. Individual calculative units (elements) of evolution. Parts a–d show the individual calculative units comprising an open pipeline of biological innovation (Figs. 1, 2). **a** The least calculative unit of multiscalar evolution. **b** A calculative unit of multiscalar biofilm evolution. **c** The least calculative unit of monoscalar evolution. Monoscalar evolution is analogous to multiscalar evolution, with the exception that the two evolving structures are neighbors, rather than being nested within one another. **d** A calculative unit of monoscalar biofilm evolution

structure is classified into four categories: primary structure, secondary structure, tertiary structure, and quaternary structure. Primary structure refers to the linear sequence of amino acids that make up the peptide chain, while secondary structure is the three-dimensional alpha helix of the peptide chain. Tertiary structure is the three-dimensional folding of the chain and quaternary structure refers to the aggregation of separate peptide subunits through ionic bonding, disulfide bridges, etc.

Primary structure can be modified through the recombination of a surrogate, the potential surrogates being the DNA blueprint itself or its RNA transcript. Tertiary structure is modified by folding that occurs spontaneously or through the action of chaperon proteins that favor a specific configuration [21, 22]. An example of innovation at the level of protein-folding is the prion [32], the agent of mad cow disease. It is a folded version of a normal brain protein that causes other native brain proteins to refold and match the structure of the prion. In this way, the folding geometry of the prion proliferates, although it is encoded only by the geometry of its own structure.

Mechanisms for restructuring cells

In 1967, Lynn Margulis published her first statement of serial endosymbiosis theory [29]. The idea was that the primary substructures of eukaryotic cells were not organelles but endosymbiotic prokaryotic cells (bacteria) that inhabited eukaryotic cells. It is now known that chloroplasts, mitochondria, hydrogenosomes, and other cell structures are in fact endosymbiotic prokaryotes or their descendants. This process of cell formation and evolution by serial endosymbiosis is referred to as symbiogenesis. Symbiogenesis was originally envisioned as the nesting of cells within cells. However, the cells of

the citrus mealybug (*Planococcus citri*) embody a hierarchy with three levels of nesting [23, 34]. The cells of a γ-type proteobacterium inhabit the cells of a β-subdivision proteobacterium, which in turn inhabit the eukaryotic cells of the insect. While the mitochondria of the insect are passed from mother to child through cytoplasmic inheritance, the nested proteobacteria (symbiotic spheres) are released from maternal cells and then re-engulfed by specialized cells within the embryo during the early stages of embryonic development. Thus, through the endosymbiosis of prokaryotic cells, the subcellular structure of eukaryotic organisms emerges.

Mechanisms for restructuring communities

When microorganisms grow as communities, their habitat range is often greater than their range as individuals [8, 9]. The lichens are a well known example; and a lichen association consists of one or more species of algae, fungi, and cyanobacteria [25]. Their habitat range as individuals is entirely different than the range of the lichen association as a whole. The structural integrity and diversity of lichen associations is so great that each has a unique genus and species designation, although they have not become speciated in the sense that plants and animals are speciated (through genetic recombination within an isolated gene pool, with genetic isolation being maintained through sexual reproductive mechanisms). Other examples of microbial communities include biofilms, bioaggregates, plaque, and aufwuchs.

The restructuring of microbial communities results from both the emigration and immigration of individual microbial cells and the emigration and immigration of cell aggregates. The geometric structure of a microbial community includes both its species composition (community structure) and the density and distribution of cells within the community (community architecture). The structure and architecture of a community result, in part, from the effects of adhesins that cause the coaggregation of specific microbial cells, thus generating specific geometric structures. Community structure is also determined by microbial behavior and quorum sensing, as mediated through homoserine lactones and related compounds. Many microbial communities also control their structure through the production of antibiotics and/or bacteriocins that exclude some organisms from community membership. In addition, the production of exopolymers is often necessary to stabilize the association by maintaining the spatial positioning of the association as a whole and by providing pores and channels in aquatic biofilms and bioaggregates.

Community structure is heritable, when the lifetime of specific community structures exceeds the generation time of the proliferating organisms, cells, and molecules that inhabit it. In this sense, DNA inherits community structure when it proliferates, just as the community inherits DNA structure when it proliferates (if the lifetime of specific genetic structures exceeds the doubling time for the community structures that it inhabits). Neither DNA structure nor community structure is inherited without variation and it is through these variations that innovative information emerges and then travels bidirectionally between DNA structure and RNA structure, as shown in Figs. 1, 2, and 3. An example of the inheritance of community structure is the death of a biofilm treated with a chemical biocide. In this situation, the organisms within the biofilm are killed but the exopolymer structure of the biofilm remains. Consequently, regrowth of the biofilm community occurs more rapidly than the colonization of a clean surface, due to the presence of protective exopolymers which also serve as inherited geometric templates. Just as the three-dimensional structure of a prion is inherited and then impressed upon unfolded brain proteins, the three-dimensional structure of a biofilm community is impressed upon newly developing biofilm communities by the presence of exopolymer from previous communities.

Mechanisms for restructuring neighboring structures versus nested structures

Figure 3a shows the smallest functional unit of multiscalar evolution. It consists of one geometric element nested within another, both structures being capable of spontaneous changes in geometric configuration and able to proliferate either individually or as part of a larger structure. The internal structure can be thought of as the inhabitant (the one nested within) and the other can be considered the habitat (the one wherein the other is nested). A change in the geometry of one affects the rate of proliferation of the other. Each calculative unit is normally part of an extended hierarchical series of nested structures, as shown in Figs. 1 and 2.

DNA, RNA, and protein molecules are neighboring structures, as opposed to being nested structures. This is to say that they exist side by side on roughly the same spatial scale. For example, DNA might not normally be a component or inhabitant of RNA nor might RNA normally be a component or inhabitant of DNA. However, both DNA and RNA are inhabitants (proliferating components) of cells and communities. Consequently, DNA and RNA can be said to be neighboring structures (with respect to one another) and, as such, their form and function can be calculated as shown in Fig. 3c, d.

Depending on the degree of nesting, the number of calculative units in which restructuring occurs within neighboring structures can be relatively small, compared with the number of units in which restructuring occurs within nested structures. Neighboring molecules are also the proliferating inhabitants of cells, which in turn are the proliferating inhabitants of communities, which are the proliferating inhabitants of ecosystems. Thus, all objects within a biofilm or bioaggregate proliferate within objects that are also proliferating. Each additional layer of nesting adds a complete set of calculative

units for each set of neighboring objects nested within. It is thus the nesting of proliferating objects that normally provides the greatest number of calculative units and that therefore is the primary determinant of emergent structures (forms) and functions (behaviors), the characteristics of each being a compromise with the characteristics of others, so that the entire matrix expands uniformly without one level of the matrix becoming a cancer that unnecessarily impedes or collapses other levels of organization. The calculative nature of this nested aspect of reality seems to have been neglected by much of twentieth century natural science. As stated by Margulis [29], "many circumstances conspire to extinguish scientific discoveries, especially those that cause discomfort about our culture's sacred norms." In time, it may be necessary to review the whole of natural science in this new light.

Mechanisms for restructuring via surrogates

Each structure involved in the geometric calculation of form and function has a life of its own in terms of being able to proliferate by itself or as part of a larger object. Each object is also able to restructure either independently or through the recombination of a surrogate, in the sense that the recombination of DNA is a surrogate for the recombination of primary structure in protein and RNA.

In this scheme of things, DNA is no longer the sole source of innovation and information. However, it does serve a somewhat unique function as a template that strongly influences the structure of RNA, proteins, cells, organisms, communities, etc.; and, as such, it is a surrogate for the recombination of the structures in which it is nested (cells, communities, ecosystems) and for neighboring structures (RNA, protein). For example, it is possible for proteins to spontaneously and independently vary in their tertiary and quaternary structure due to folding and aggregation. However, is not possible for them to vary in terms of their primary structure (amino acid sequence) or secondary structure (alpha helix), except through the reshuffling of DNA molecules coding for their sequence of amino acids or through the reshuffling of mRNA molecules via the action of spliceosomes. Thus, nucleic acids that serve as surrogates for proteins allow a restructuring of protein that would not occur spontaneously.

However, certain other evolutionary events (e.g. the emergence of non-reducible complexity) could not otherwise occur without restructuring first at the community, cellular, or protein level. If beneficial, these higher-level recombinational events can then become encoded as genetic sequencing after the effect of the structural change has already been manifested as increased rates of proliferation. Endosymbiosis is one of the best examples. In this situation, DNA recombination becomes constrained by a spontaneous change in cell geometry resulting from the assimilation of an endosymbiont. If the endosymbiosis is essential for survival in some environments, then the environmental conditions in those environments constrain the retention of recombinant host DNA to sequences that code for enhancement and retention of the endosymbiont, while also eliminating any prior or novel genetic programming that might interfere with endosymbiosis. This is in addition to the possibility of direct migration of DNA fragments from endosymbiont to host. As discussed in detail elsewhere [3], the effect of DNA on the proliferation of the endosymbiosis occurs through events within the internal environment of the association (the expression of nucleic acid geometry through differential transcription and translation), while the effect of the endosymbiosis on the proliferation of DNA occurs through larger events within the external environment of the endosymbiosis (the expression of endosymbiosis geometry through differential proliferation).

Hypolimnia and the emergence of the *Chlorochromatium aggregatum* consortium

A consortium is a small bioaggregate (cell aggregate) consisting of two or more prokaryotic bacterial species arranged in a well-defined and highly reproducible geometric pattern. Bacterial consortia are commonly found within lakes that become thermally stratified, with low-density (warm) water at the surface and high-density (cool) water at the bottom. Solar radiation contributes to stratification by causing surface warming, while wind action produces waves that tend to destratify by causing circulation. Consequently, the upper waters of the epilimnion are mixed and aerated, while the lower waters of the hypolimnion tend to be stagnant and anaerobic. In this situation, three layered bacterial communities form along the physicochemical gradients that occur within the hypolimnion [6, 7, 37]. All three communities consist of obligate anaerobes. The upper layer is red, due to purple photosynthetic bacteria, the next is green, due green photosynthetic bacteria, and the lowest is colorless, due to sulfate-reducing bacteria and other heterotrophs. All three of these primary bacterial layers (plates) consists of sublayers, each containing a unique community of prokaryotic organisms.

One unit of calculative feedback lies between the structure of the physicochemical gradients and the structure of the communities. Changes in the structure of the physicochemical gradients within the water column affect the proliferation of these three-layered microbial communities. Changes in the structure of the communities affect the proliferation of the physicochemical gradients (i.e. the growth of microbial communities affects the rate of expansion of hypolimnetic gradients by modifying the quantity and quality of light penetrating the water column, by expanding the anoxic zone through oxygen consumption, by modifying sulfide gradients through sulfide production, and by modifying thermal gradients through the absorption of solar

radiation). This calculative element links the flow of biological information (innovation) to the flow of physicochemical information (innovation) at the ecosystem level.

In autumn, surface waters gradually cool until they are more dense than the waters in the hypolimnion. This mixes the water column and introduces oxygen from top to bottom. The oxygen causes the obligate anaerobes inhabiting the hypolimnion to clump and form macroscopic aggregates in which the cells in the center of the aggregates are protected from oxygen toxicity. This effect can also be induced artificially during summer stratification. Anaerobic samples from the hypolimnion remain turbid and dispersed if they are collected anaerobically, but aggregate if they are collected aerobically (leaving large clumps and strands several centimeters in size suspended in a clear solution). Adding sodium sulfide or other reductants to aerobically collected samples causes the dispersal of clumps and restores the turbid suspension of planktonic cells.

One of the bacterial species within the layered bacterial communities of hypolimnia is the *Chlorochromatium aggregatum* consortium [7]. The geometry of the association is shown in Fig. 4. As in the case for lichens, it is an association consisting of more than one organism but is still given its own genus and species designation, due to the unique and stable arrangement of cells within the association in terms of size, geometry, and composition. It is one of many such bacterial associations found in the hypolimnion and referred to as consortia. Within the *C. aggregatum* consortium, there are two bacteria. One is photosynthetic and the other is heterotrophic. The heterotrophic associate was originally thought to be a colorless central sulfate reducer surrounded by green phototrophs [31], although recent genetic studies contradict this [30]. If there are alternative associates capable of filling the heterotrophic niche, then some might be sulfate reducers, while others are not.

The phototroph generates sulfate and dissolved organic matter, while consuming sulfide and carbon dioxide. If the central heterotroph is a sulfate-reducer, then its metabolism complements that of the phototroph by generating carbon dioxide and hydrogen sulfide while consuming organic matter and sulfate. If it is not a sulfate-reducer, then the metabolism of phototroph and heterotroph is balanced with regard to carbon but not sulfur. In addition, the phototrophic associate is the only phototroph in the hypolimnion that lacks the gas vesicles required for positioning along the vertical chemical and light gradients of hypolimnia. Cells synthesize more gas vesicles in order to rise and make fewer in order to descend. However, *C. aggregatum* is the exception. The heterotroph is flagellated and thus there is no need for the phototroph to produce gas vesicles in order to position itself. Presumably, the calculative geometric feedbacks of nested proliferation have reprogrammed the heterotroph to position itself so as to meet the needs of the phototroph. These feedbacks would be horizontal, between recombination of heterotroph DNA and phototroph DNA (feedbacks between neighboring structures, as opposed to nested structures), changes in the geometry of each affecting the proliferation of the other and calculating compromises between the form, function, and proliferation of each.

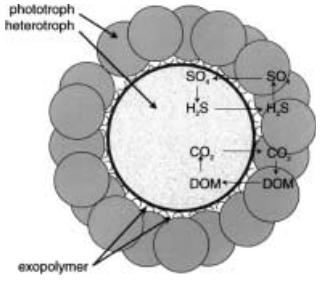

Fig. 4. The *Chlorochromatium aggregatum* consortium

Innovation also arises within the *C. aggregatum* consortium through vertical feedbacks between the geometry of the proliferating consortia and the geometry of the proliferating DNA that resides within each consortium. For example, the arrangement of cells within the *Pelochromatium roseum* consortium (red version) is identical to the *C. aggregatum* consortium (green version), except that the green phototroph is replaced by a red-pigmented phototroph. In this case, the association evolves through the recombination of red and green cells within the consortium, the calculative element being the reciprocal effect of changes in the structure of the consortium on the proliferation rate of genetic structures and of changes in genetic structure on the proliferation rate of the consortium. The red version of the consortium contains carotenoids that protect it from photooxidation and thus it originates in a hypolimnetic bacterial layer (plate) located immediately above a layer containing the analogous green version. The upper hypolimnetic layer containing the red consortium thus shields the lower layer containing the green consortium. Consequently, the red carotenoids function both at the cellular and ecosystem level. This relationship is somewhat analogous to the dual set of red and green chromophores in *Euglena rubra*. This alga occurs as part of the epineuston community (just above the air–water interface on the surface of lakes and ponds). Solar radiation is extremely intense in this situation and a red set of chromophores migrates to the periphery of the algal cells at midday when the sun is most intense (shielding the green set from the light), while the green set migrates to the periphery in the late afternoon as the light intensity decreases. This red to green color change can also be induced artificially by shading the red cells. In this situation, as in the hypolimnion, the protective red carotenoids are functional at the cell, community, and ecosystem levels of organization.

The *C. aggregatum* consortium is also a subcomponent of a larger system of cellular reshuffling or recombination that occurs seasonally when lakes mix in the

autumn. Innovation arises through the recombination of cells when the hypolimnetic anaerobes aggregate, protecting themselves from oxygen as the aerobic water from the epilimnion mixes with the anaerobic waters of the hypolimnion. All cells present tend to be trapped within these aggregates and this leads to countless coincidental community structures that either settle to the anaerobic sediments of the lake after ice cover, or that are carried elsewhere by waterfowl. When strict anaerobic conditions again begin to return to the water column, these somewhat coincidental, synthetic anaerobic communities begin to disperse, expand, and grow, forming the layered bacterial plates that are characteristic of anaerobic hypolimnia during summer stratification. This annual pattern of bacterial aggregation and dispersal, within and among lakes, results in the reshuffling of cells within communities, the reshuffling of whole communities within hypolimnetic ecosystems, and the reshuffling of whole hypolimnetic ecosystems between lake ecosystems. This creates numerous calculative feedback mechanisms.

Epilimnia and the emergence of planktonic bioaggregates

In the epilimnion of eutrophic lakes, bioaggregates commonly consist of associations between heterotrophic bacteria and cyanobacteria [1] arranged in varying geometric patterns, normally with the heterotroph on the exterior and the phototroph on the interior. Cyanobacterial production of oxygen and consumption of carbon dioxide is balanced by bacterial consumption of oxygen and production of carbon dioxide [33]. This is analogous to the metabolic balance between anaerobic phototrophs and heterotrophs within *C. aggregatum* (discussed in the previous section).

The geometry of a bioaggregate, consisting of the cyanobacterium *Microcystis aeruginosa* and its associated heterotrophic bacteria, is shown in Fig. 5. In this situation, heterotrophs become embedded in exopolymers produced by *M. aeruginosa*, either as parallel chains or somewhat randomly. In some cases, the association is highly ordered, as shown here, with chains of rod-shaped heterotrophs embedded within radiating strands of *Microcystis* spp exopolymer. In other situations, a variety of heterotrophs is randomly distributed within exopolymer, or the hetrotrophs are entirely absent.

In this situation, the community structure of the bioaggregate changes through cell immigration and emigration to and from the community. The resulting changes in the community structure of the bioaggregate affect the proliferation of the genetic structures (DNA sequences) within. Similarly, the genetic structures change through genetic recombination and then affect the proliferation of community structures. This then provides a multiscalar calculative unit sufficient to function as one of many elements in the calculation of

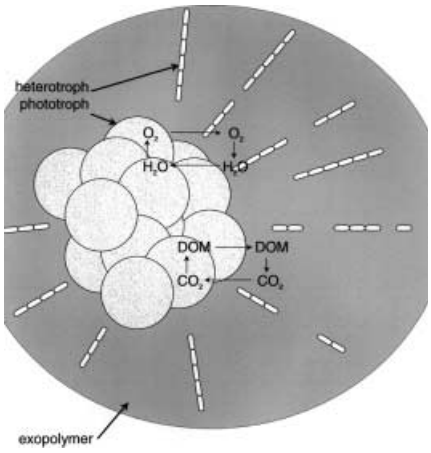

Fig. 5. Bioaggregates of *Microcystis* spp and associated heterotrophic bacteria. The association between *Microcystis* spp (cyanobacteria) and heterotrophic bacteria results in bioaggregates that vary in size from clusters of a few cells to aggregates several millimeters in diameter

compromises between the form of proliferating DNA sequences and the form of the proliferating bioaggregate communities in which they are nested. As in the case of the *C. aggregatum* consortia, the transport of bioaggregate communities from one lake to another via waterfowl creates an additional calculative unit at the next layer of nesting, between the epilimnetic ecosystem and microbial bioaggregate communities.

Lotic environments and the emergence of surface-associated biofilms

Planktonic bioaggregates and consortia are commonly suspended in static or slow-moving waters (lentic environments). Many microbial biofilms are layers of microorganisms colonizing surfaces; and these require water movement within the hydrodynamic boundary layers of surface microenvironments, to continually resupply substrates and remove metabolic waste [5]. Thus, the growth rate of surface-associated microcolonies can be independent of substrate concentration, while being highly dependent upon laminar flow velocities within the surface microenvironment [4]. For example, the exopolymers of herbicide-degrading biofilms sorb herbicide from flowing solutions. Once the substrate has been concentrated in exopolymer, it is then assimilated and degraded [35].

As in the case of planktonic bioaggregates and consortia, changes in the genetic structure of biofilms affect the proliferation of the biofilm community and changes in community structure affect the proliferation of nucleic acids. Changes in community structure through emigration and immigration can involve a wide variety of microbial behaviors [26]. In some strains of bacteria, one of the two progeny is always shed from the biofilm after cell division (the shedding maneuver). In other strains, the progeny form a tightly packed monolayer on the

surface with little or no shedding (the packing maneuver), while some strains form a monolayer but glide away from one another, forming a dispersed cell monolayer with little shedding (the spreading maneuver).

While individual members of biofilm communities normally reproduce by binary fission and can create new biofilms by colonization and succession, biofilm communities can also propagate by sloughing. This results from outward growth of the biofilm away from the solid substratum and into the hydrodynamic boundary layer, until a fragile lobe is formed and the mass of cells is eventually sheared away from the parental biofilm. If the mass of sloughed cells adheres to a suitable surface, it continues to grow by expanding outward across the surface. This sloughed mass then becomes an ecosystem-level recombination portal (as shown in Fig. 1). That is to say that entire communities of cells are sheared away from mature biofilm ecosystems and recombined at random to form hybrid ecosystems, complete with predators, parasites, well defined boundaries, and internal nutrient cycling. Changes in the structure of microbial ecosystems (due to the shearing of community structures from biofilms and the subsequent aggregation of sheared structures) affect the proliferation rate of communities; and changes in the structure of communities (through the emigration and immigration of individual cell lines) affect the proliferation rate of microbial ecosystems. This again provides another least calculative unit necessary for multiscalar evolution by nested proliferation. This includes two proliferating geometric structures, one nested within the other and both capable of restructuring in a somewhat random way. The feedback between the two provides a minimal calculative mechanism responsible for computing compromises between the features of the proliferating objects.

Summary and conclusions

The organelles of eukaryotic cells were derived from endosymbiotic bacteria [29] and the concept of serial endosymbiosis was first published in a 1967 paper titled ''Origin of mitosing cells'' in the Journal of Theoretical Biology. At nearly the same time, it was suggested that biological innovation must flow from the cell to DNA, rather than exclusively from DNA [11, 12, 13, 14, 15, 16] to the cell; and it now appears likely that ''DNA did not create life; life created DNA'' or, in other words, ''DNA is a mechanism created by the cell to store information produced by the cell.'' [12, 13, 15, 17]. More recently, it was also thought likely that innovation flows from community structure to DNA structure [3, 8] . If so, then one might say that DNA is a mechanism used by the community to store information produced by the community. This would account for the emergence of biofilms, bioaggregates, and microbial consortia in a calculative manner that is somewhat analogous to the emergence of prokaryotic and eukaryotic cells.

Calculative feedbacks between nested geometries make it possible to envision evolution in terms of a computer metaphor (i.e. the analogy between a computer and a self-programming universe that encodes information in the space–time positioning of each physical, chemical, and biological object), as opposed to the agricultural metaphor used by Charles Darwin [20] (i.e. the analogy between natural selection and the domestication of livestock and crops by selective breeding). The difficulty with the agricultural metaphor is that if all forms of information reside in context as well as in content [3], then information cannot be isolated and selected in the same way that a farmer might select one tomato plant and discard another. Attempting to do so might eventually come to be regarded as a form of superstition. As an alternative, ''let us imagine the structure of a community in terms of a message, written in a language with a number of symbols equal to the number of species and where individual symbols stand for individuals'', as suggested by Margalef [28] at nearly the same time that Margulis and Commoner first discerned the origins of cell structure and biochemistry.

A second difficulty with the agricultural metaphor is that, in some situations, such as the growth of microorganisms in continuous culture [3, 9], modification (evolution) can occur without selection [20, 36]. In this case, organisms are lost by attrition and are not lost by selective death or selective removal, as is the case with selective breeding. The computational metaphor thus makes it possible to think more precisely by discriminating between differential growth and differential selection rather than lumping the two together. This reserves the term ''selection'' specifically for differential death and removal, as in the strict functional sense of the agricultural metaphor. When used in the more general sense, ''selection'' becomes a misnomer. It could be argued that this is a matter of semantics and not science. It could also be said that, as scientists, we do not think, we do experiments. However, this might be a mistake. As language progresses, it gradually makes finer distinctions and these are necessary to formulate more insightful experiments. This is an essential part of the process by which logical thought evolves through feedbacks between empirical experiments (experimental research) and thought experiments (theoretical research). Without both, there can be no calculative unit.

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