The origin of cellular life

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Summary

This essay presents a scenario of the origin of life that is based on analysis of biological architecture and mechanical design at the microstructural level. My thesis is that the same architectural and energetic constraints that shape cells today also guided the evolution of the first cells and that the molecular scaffolds that support solid-phase biochemistry in modern cells represent living microfossils of past life forms. This concept emerged from the discovery that cells mechanically stabilize themselves using tensegrity architecture and that these same building rules guide hierarchical self-assembly at all size scales (*Sci Amer* 278:48–57;1998). When combined with other fundamental design principles (e.g., energy minimization, topological constraints, structural hierarchies, autocatalytic sets, solid-state biochemistry), tensegrity provides a physical basis to explain how atomic and molecular elements progressively self-assembled to create hierarchical structures with increasingly complex functions, including living cells that can self-reproduce. *BioEssays* 22:1160–1170, 2000.

Introduction

My premise in this article is that evolution is the process by which matter self-organizes in space and, thus, that the origin of life is merely one aspect of the natural evolution of the cosmos. The only difference between living organisms and inorganic matter composed of the same atoms (carbon, hydrogen, nitrogen, oxygen, phosphorus and sulfur) is how these atoms are arranged in three-dimensional (3D) space. Indeed, the properties of all matter are ensemble behaviors that emerge from collective interactions among different components (e.g., a single atom does not exhibit a melting point). Thus, the 3D spatial relationships between the parts within a material govern how it will behave and whether or not it will self-assemble with other components to build more complex hierarchical structures. From this perspective, the problem of how life first originated distills down to a question in architecture.

My thesis is that understanding the principles that guide how atomic and molecular components progressively self-organize in 3D to form the microscopic structural hierarchy that comprises the living cell—the most basic unit of life—may provide new insight into how life first originated on this planet.

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In this article, I will describe a set of design principles that govern how cells structure themselves at the microscopic level and which appear to guide the hierarchical self-assembly of natural structures from atomic to macroscopic scales. I will then present a scenario of how the first cells might have originated in light of these architectural and energetic constraints. In the process, I hope to show that the evolution of inorganic matter into organic structures that self-replicate, including living cells, is inevitable given sufficient time.

The architecture of life

My research laboratory focuses on how eukaryotic cells control their shape and function. We discovered that cells mechanically stabilize themselves through use of "tensegrity",[1–6] an architectural form that comes from the R. Buckminster Fuller world of geodesic architecture.[7] Tensegrity systems gain their mechanical stability from continuous tension and local compression; there are two classes, prestressed and geodesic[1,5] (Fig. 1). Prestressed tensegrity structures maintain shape stability within a tensed network by incorporating other support elements that resist compression; their stability depends on the prestress (pre-existing tension) in the structure before application of an external load. When externally stressed, the discrete structural elements move and change their orientation relative to one another until a new equilibrium configuration is attained; the larger initial prestress, the stiffer the structure.[6] Geodesic tensegrity structures are composed of stiff struts, all arranged geodesically (e.g., hexagons and pentagons in a geodesic dome) and, by definition, each mapping out a "minimal path" between two joints in the network. Each stiff strut may resist *either* tension or compression at a particular location; however, only tension is transmitted continuously throughout the system.[5] Importantly, the mechanical properties of both types of structures are ensemble behaviors: they *emerge* from collective behavior among the interacting elements.[3,6] Hierarchical tensegrity structures also may be created using smaller tensegrity networks as building elements;[1,3–5,7] these exhibit even greater structural efficiency as well as coordination between part and whole.

Living organisms use tensegrity at all size scales. Think of the human body: it stabilizes its shape by interconnecting multiple compression-resistant bones with a continuous series of tensile muscles, tendons and ligaments, and its stiffness varies depending on the tone (prestress) in its muscles. By interconnecting multiple smaller tensegrity subsystems, we maintain overall stability of our whole body (a structural

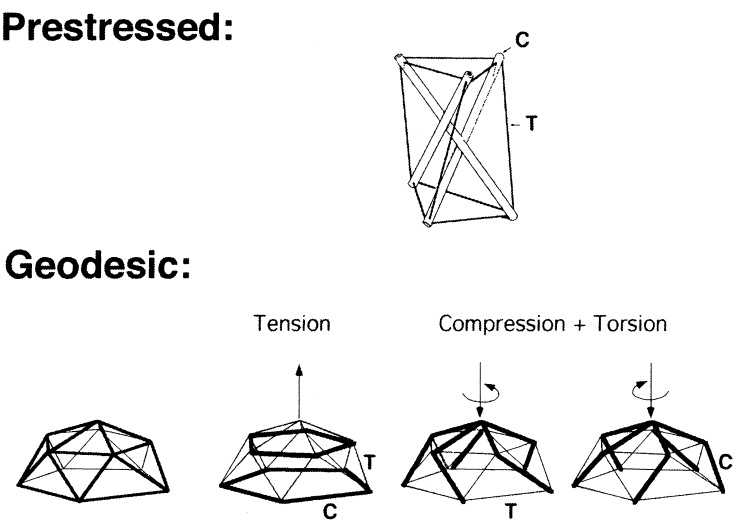

Figure 1. Prestressed and geodesic forms of tensegrity structures. In the prestressed structures, the large white struts always bear compression (C) while the continuous series of thin black cables bear tension (T). The stiff black struts that constitute the geodesic structures either bear tension (T; thin lines) or compression (C, thick lines), depending on loading conditions (as indicated by the large arrows), however, only tension is continuous.

hierarchy) while destabilizing an individual module (e.g., lifting one leg). Similar building rules are used in all animal bodies, including insects, and in plants.[1,8]

Tensegrity also applies at the single-cell level. Contractile actomyosin filaments in the cytoskeleton of eukaryotic cells generate a stabilizing tensile prestress, which is resisted by other cytoskeletal filaments that resist compression (e.g., microtubules and cross-linked microfilament bundles) and by external adhesion tethers.[1–4] Prokaryotic cells contain a primitive filamentous cytoskeleton[9,10] and similarly use molecular motors to generate tensional forces that drive changes in bacterial cell shape during division.[11] The eukaryotic cell is also a hierarchical tensegrity structure since its subcellular components (e.g., mitotic spindle, organelles, molecular filaments) use similar building rules[1,2,12–14] and geodesic structures can be observed at all size scales, including enzyme complexes, viruses, transport vesicles, actin geodomes, and the submembranous cytoskeleton.[1,2,15]

The important point in the present context is that similar geodesic forms existed in the inorganic world of crystals, water molecules and Buckminsterfullerenes (60 carbon-based geodesic spheres) long before DNA ever came into existence. These observations emphasize that, while changes in DNA may generate biological diversity, genes are merely one product of evolution and not its driving force. But what is the driving force? Perhaps understanding why Nature uses tensegrity might help to solve this riddle.

Fundamental principles of natural design

Energy minimization, spatial constraints and tensegrity

Local physical force interactions are responsible, when satisfying the fundamental need to minimize energy in a system, for the establishment of a mechanical equilibrium between interacting parts and hence, shape stability on all size scales.[16,17] However, topological rules also must be satisfied. A tesselation of hexagons, whether composed of carbon atoms or ceramic tiles, will always form a plane. Incorporation of a few pentagons in the hexagonal array results in formation of geodesic domes and spheres whether composed of carbon, proteins, or aluminum struts. Similarly, each polyhedral subunit within any closely packed 3D foam will on average contain 14 sides whether composed of soap films, metal grains or living cells. Moreover, the edges of these polyhedra will meet in groups of four at an angle of 109.5 degrees, the same fundamental angle found in crystal lattices, in bonds between hydrogen and oxygen nuclei in water, and in carbon bonds that form the basis for organic life. Thus, it is the combination of these topological and energetic constraints that drives progressive self-assembly and hence, biological evolution (Table 1).

During self-assembly, attractive and repulsive forces (tension and compression) must come into balance to create stable forms or else the material would expand or shrink endlessly. The most efficient self-stabilizing structures will be dominated by tension members because if the members are made thinner to minimize weight or to span large distances, compression members will yield first due to bending or buckling failure.[8,16] Tensegrity systems that maximize tension elements and minimize those in compression while establishing a mechanical equilibrium therefore use less mass to maintain shape stability and hence, minimize associated energy (and metabolic) costs.

The 3D form that any structural network exhibits is determined by the material properties of its members, their arrangement, and any free movement that may exist in the joints. One way to stabilize the joints is to increase the load-

Table 1. Natural design principles

- ***Minimize energy expenditure.*** Nature is most economical and, thus, all natural structures tend to minimize energy and mass.
- ***Obey spatial constraints.*** Certain topologial rules constrain the possible forms that matter can take on, regardless of size or position.
- ***Develop emergent properties through architecture.*** At every size scale, complex properties and functions emerge from the behavior of the ensemble; the material properties of any single element is much less important than how the different elements are joined and positioned in 3D.
- ***Establish a mechanical equilibrium.*** Architectural stability requires the establishment of a global balance of mechanical forces, although local regions may be stressed.
- ***Use discrete networks.*** Nature does not use bulk solids to build; discrete porous networks offer greater structural efficiency and versatility.
- ***Maximize tensile materials.*** Disproportionate use of compression elements puts greater demands on energy (and eventually metabolism) for their production, support and movement as the relative distance between interacting components increase.
- ***Stabilize through triangulation or prestress.*** Prestress and triangulation provide more efficient ways to stabilize discrete networks. Triangulation results in stiff structures whereas prestress provides both flexibility and strength.
- ***Use structural hierarchies.*** Structural efficiency is maximized and evolution accelerated through the use of hierarchical networks, which are themselves discrete structures on a smaller scale.
- ***Develop self-renewing functional webs through emergence of autocatalytic sets.*** Once molecules with catalytic functions appear, self-reinforcing webs of chemical and structural interactions will spontaneously come into existence and expand at the expense of other non-interactive components.
- ***Enhance functional efficiency through solid-state biochemistry.*** Most of the chemical and enzymatic functions carried out by living systems proceed on insoluble scaffolds using solid-phase catalysis. This increases the efficiency of chemical reactions, stabilizes functional networks, and allows entire metabolic systems to self-assemble with others to create hierarchical structures with enhanced functionality.

bearing materials in these regions (e.g., triangular supports in corners of rectangular picture frames). A more efficient way to stabilize a rigid framework is through triangulation such that each strut is oriented so as to constrain the joint to a fixed position. For these reasons, Nature repeatedly selected out geodesic tensegrity systems. A more elegant and economical solution to this problem that results in both structural efficiency and flexibility is to impose an internal tension (prestress) to reduce the play in the joints. Hence, Nature soon discovered prestressed tensegrity structures as well (Table 1).

In summary, because it is the most economical solution to the design challenges created by spatial constraints, energy minimization rules, and the need to efficiently balance forces, tensegrity is the way Nature builds. It is the only building principle that can provide a single explanation (Occam's razor) for how both unicellular organisms with geodesic exoskeletons (e.g., radiolaria) and huge behemoths with prestressed musculoskeletons (e.g., dinosaurs) could ever come into existence.

Autocatalytic sets and solid-state biochemistry

Another concept that may be relevant to how life originated is the idea is that once structures come about that can mediate catalysis, then coherent self-reinforcing webs of chemical reactions or "autocatalytic sets" will spontaneously emerge.[18] For example, if a primitive catalyst (protoenzyme) 1 accelerated the formation of protoenzyme 2 and 2 accelerated the formation of 3, and so on, then at some point protoenzyme X would emerge that could catalyze formation of protoenzyme 1. Loop closure would result in self-reinforcement of this particular web of interactions. Thus, this particular cluster of interacting components would increase in abundance relative to molecules that were excluded from the web (Table 1).

On one hand, if autocatalytic sets were to form in solution (as commonly assumed), then huge numbers of reactions may be required before self-organization would be observed. On the other hand, if some of these primitive protoenzymes catalyzed the assembly of 3D scaffolds that bound the interacting molecules and brought them in close proximity, then the likelihood that the product of one reaction could function as a substrate for a neighboring enzyme would increase dramatically. Incorporation of this form of solid-phase or "solid-state" biochemistry would therefore greatly increase the probability that an autocatalytic set would develop and be sustained over time (Table 1).

In fact, one of the major limitations of past theories of the origin of life is that they failed to consider the importance of solid-state biochemistry. The cytoskeleton of eukaryotic cells orients most of the cell's organelles and many of the enzymes and substrates that make up its metabolic machinery.[14,19,20] This increases the efficiency of chemical reactions because substrate availability is not diffusion-limited in this context and much larger molecules that would normally be insoluble or spatially restricted by diffusion may be funneled into complex metabolic pathways.[21] It also provides a mechanism to integrate structure and function such that cells can respond directly to environmental stresses (and scaffold deformation) by altering cellular biochemistry[22–24] and strengthening the scaffold against disruption.[3] Importantly, prokaryotic cells also appear to use solid-state biochemistry on primitive

cytoskeletal scaffolds to carry out their metabolic functions.[9,10,25]

Structural hierarchies

Natural architectural design is dominated by use of structural hierarchies.[1,26] The discrete molecular networks that constitute natural materials exhibit structures on several size scales and the smaller elements themselves are composed of subcomponents that display specialized microarchitecture. The existence of hierarchical networks optimizes their structural efficiency[26] and their surface area available for interacting with the environment, while simultaneously minimizing the amount of building materials, energy expended, and metabolic cost.[5] Hierarchical organization also leads to novel forms of control: the higher level network constrains the motion and hence, position or reactivity of the smaller subcomponents.[17] Each system-level jump in hierarchical assembly therefore results from a "symmetry-breaking" event in which the potential degrees of freedom of each element decrease as a result of their placement within a 3D ensemble that constrains their motion. Thus, it is no surprise that symmetry breaking is a hallmark of pattern formation in all systems and at all size scales.[27]

The existence of hierarchical organization in matter may be an absolute requirement for the evolution of life. Simple probability analysis reveals that, if matter were to self-assemble in a hierarchical manner, then evolution would be greatly accelerated. Take the following published example:[28] the goal is to assemble a watch containing thousands of parts. There is some probability that this process may be frequently interrupted over time such that any set of elements that does not yet form a stable system will fall apart completely. A worker who organizes his operations into a sequence of independently stable subassemblies will build that complex system in a much faster time than another who simply joins one part to another sequentially. Hierarchies will therefore evolve more rapidly than non-hierarchical systems containing the same number of components (Table 1). This analysis also reveals that it takes no more time to develop the three highest order levels of a hierarchical structure than it does to create the three lowest.[28]

Once progressive hierarchical self-assembly begins, it is likely to explore many more potential structural configurations than if sequentially assembled and hence result in the creation of structures with more highly developed forms and functions. Use of multiple smaller networks that independently self-stabilize also would likely be favored by natural (environmental) selection since the function of the whole is not necessarily compromised by loss of a part. This would promote morphological diversification and permit self-repair on smaller size scales without loss of pattern integrity of the whole, key features of living systems. Hierarchical structures would therefore exhibit increased stability, thereby promoting their survival and their ability to undergo further self-assembly. Hierarchical tensegrity systems, which display harmonic coupling (vibrational information transfer) between distant elements and coordination between part and whole,[2,29] would offer additional advantages for integrating structure and function in evolving life forms.

The origin of the living cell

Let us now explore whether the building rules described above (Table 1) can help to explain the progressive evolution of the hierarchy of life, from inorganic part to whole living cell. An overview of this scenario is presented in Table 2.

From atom to crystal

Atoms created from the cosmic dust exhibited charges and mass densities that led to their self-assembly into closely packed arrays or crystal lattices.[17] The stability of these structures resulted from a balance between strong attractive (tensile) electron-bonding forces and the ability of the relatively dense nucleus of each atom to resist being compressed. Crystals are rigid because the energy penalty for wrong neighbors among the atoms is extremely high and geodesic forms predominate. In response to environmental stresses, these lattices reversibly deform up to a point; high stresses produce local lattice imperfections (dislocations), which propagate and result in irreversible changes in lattice topology. Self-assembly of new and potentially more complex crystal forms preferentially occurs in these zones of dislocation. In other words, mechanical strain-induced changes in topology accelerate or "catalyze" the formation of new structural arrangements within these rigid networks. Furthermore, once nucleation sites arise, patches of new structure (whether due to addition of the same or different atoms) extend by accretion at a rate that depends on how quickly the parts at the interface rearrange themselves. The ability of a material to modify its structure therefore increases as the flexibility of its framework increases. For this reason, development of structural diversity in the inorganic world of rigid materials proceeded at a relatively slow pace and only a limited number of macroscopic forms were created (e.g., compare diversity of crystal types versus proteins).

From crystals to organic molecules: catalysis by clay minerals

Combinations of different types of atoms resulted in the creation of minerals with more complex structures. Clay, for example, is a porous lattice of atoms arranged geodesically within octahedral and tetrahedral forms;[30] however, unlike in rigid atomic lattices, these octahedra and tetrahedra are not closely packed. Because of decreased spatial restrictions, these tetrahedra and octahedra can move slightly and slide relative to one another without disrupting network topology. This flexibility is responsible for clay's ability to elastically

Table 2. A scenario for the origin of life

Step 1: Atoms coalesce to form crystal lattices that are rigid and geodesic. Local lattice imperfections catalyze structural remodeling and formation of new crystal structures; rate is dependent on the flexibility of the network.

Step 2: Clay minerals exhibit enhanced flexibility due to their microarchitecture, which permits them to catalyze chemical reactions with greater efficiency, including reactions that produced the first organic polymers.

Step 3: Oligonucleotides and polypeptides synthesized on clay fold into 3D structures that stabilize through the presence of an internal prestress. Use of this form of tensegrity architecture provides enhanced flexibility, which further increases catalytic efficiency, makes allosteric regulation possible, and accelerates molecular self-assembly.

Step 4: Primitive ribozymes appear that can catalyze synthesis of longer RNAs as well as proteins, in addition to self-replicating. Early polypeptides include molecules that promote correct folding of other molecules and that form scaffolds which immobilize the autocatalytic web of chemical interactions that supports the protein synthetic machinery. This form of solid-state biochemistry leads to the development of the primitive ribosome, which self-replicates when excess proteins and RNA are produced.

Step 5: Longer proteins with a greater range of functionality are produced, which take over many of the roles formerly carried out by RNAs. RNA-directed DNA polymerases appear, which produce DNA using RNAs present within primitive ribosomes as coding sequences; these DNAs physically integrate within the same RNA–protein scaffolds. DNA-directed RNA polymerases emerge, which use these DNAs to code for the primitive ribosomal RNAs and proteins. A new higher order autocatalytic set emerges: the linked translation–transcription complex.

Step 6: Production of longer DNAs, RNAs and proteins leads to structural diversification. Transcription–translation complexes with new metabolic functions emerge as well as machinery for contraction and engulfment of other functional scaffolds.

Step 7: Transcription–translation machines, express hydrophobic proteins on their outer surface, pull lipids out from the surrounding environment, thereby spontaneously forming a surface membrane enclosing their catalytic machinery. The first coupled transcription–translation membrane insertion complexes appear.

Step 8: Progressive coalescence of primitive membrane-lined cells that contained self-renewing, transcription–translation scaffolds with other cells that contained similar scaffolds with different functions results in formation of a cell containing a higher order autocatalytic web that self-reinforces its own formation. Cells that exhibit membrane ion channels and signal-sensing molecules that couple to the internal scaffolds exhibit enhanced stability. Cells with longer DNAs are selected out because of its ability to prestress and thereby stabilize the surface membrane. Cells that contain contractile machinery and engulfment mechanisms are most likely to diversify and, hence, exhibit autonomy.

Step 9: A primitive cytoskeletal mechanism appears that can coordinate the complete separation of all of the cell's self-replicated scaffolds, their attached metabolic machinery, and their surrounding membranes such that one cell could now divide into two; the first true autonomous self-reproducing cells come to life.

conform to substrates and thereby catalyze chemical reactions.

Interestingly, there is now great evidence to suggest that biological evolution began in layers of clay.[31,32] Clay can preferentially adsorb or catalyze the synthesis of many small organic molecules, including amino acids, purines, pyrimidines, and energy-rich molecules.[32–36] More importantly, clay can catalyze the polymerization of polypeptides[37,38] and oligonucleotides,[39,40] in addition to promoting its own formation. The key point here is that all of these novel functions of clay result directly from how the individual atoms are spatially arranged within its internal supporting lattice.

Because of their shallow depth, clay pools would likely contain higher concentrations of potential interacting molecules than could ever accumulate in the diffusion- and convection-dominated world of the deep oceans. Organic molecules produced in clay pools would be further supplemented by those that formed spontaneously on the young planet earth or were delivered by meteorites.[41,42] Ions and metal compounds that can act as primitive catalysts and rudimentary coenzymes would also be present.[31] Thus, this primordial pudding would be rich indeed.

At this early point in evolution, production of the first primitive macromolecules was likely based on "mass-production" by clay (functioning as a primitive solid-state catalytic scaffold) rather than on "self-reproduction". However, because the fidelity of the system was low, a great diversity of structures would be produced. The result was a combinatorial chemistry laboratory on a planetary scale: all possible combinations that might be explored and environmentally selected for extended survival (based on structural stability) would be discovered, if provided enough time and variations in environmental conditions.

Emergence of functional biomolecules

The polypeptide chains and oligonucleotides that polymerized on clay would immediately take on new 3D conformations based on the drive to minimize free energy locally.[31] Small regions of each protein's amino acid backbone would spontaneously fold into helical forms that stabilize themselves through a balance between the tensional forces due to multiple hydrogen bonds and the ability of the protein coil to resist these inward-directed (compressive) forces and hence, using tensegrity. Another peptide domain—the β chain—may have evolved later through mechanical distortion of the α chain,[31] much like the DNA helix can transition into a new stable conformation when physically extended.[43] β · chains also can self-assemble with others to form higher order pleated sheets.

Work on the evolution of the genetic code suggests that the first proteins were dominated by glycine residues, which would preferentially fold into helices or pleated sheets.[44,45] Even more interesting is that prions—protein-based infective agents that propagate in the absence of DNA or RNA—develop their infective capacity through mutations that induce an α-chain and surrounding regions to convert into two anti-parallel β-strands.[46] This small conformational change is sufficient to induce similar changes in normal prions when they self-assemble with mutant proteins, and hence trigger a cascade of molecular "self-replication". Interestingly, there is a high sequence homology between the most frequently occurring amino acid sequences in known prions and sequences preferentially formed by inorganic mechanisms of peptide formation, suggesting that these simple proteins may be remnants of an early stage of molecular evolution.[47]

Establishment of higher order tertiary structure and functionality in proteins also involves hierarchical assembly using tensegrity as a guide.[1] The small helically (or otherwise) stiffened regions of the protein are separated by parts of the same amino acid backbone that act as flexible hinges. Because of tensile hydrogen-bonding or ionic forces, these stiffened regions fold back on themselves in order to stabilize the entire molecule, thereby creating an internal prestress. The stiffened regions may be compressed locally, even though forces are equilibrated across the whole molecule. The tensile prestress becomes apparent when the peptide backbone is enzymatically cleaved; the entire protein splays open and shape stability is lost. Thus, biological evolution may have selected for stable 3D molecular configurations stabilized through tensegrity, rather than for sequence per se. This concept is supported by the fact that "superfolds", the most common of the ancient protein structural forms, can accommodate a wide variety of sequences in the absence of any detectable sequence homology.[48,49]

The first RNAs, which probably resembled a single hairpin loop region of present day tRNAs (reviewed in Ref. 50), similarly used prestress to stabilize their secondary structure. A portion of the single-stranded oligonucleotide chain folds back on itself to form a small double helical region that prestresses the whole structure such that an open loop becomes stabilized at the other end. The current form of tRNAs may have come about through self-assembly and ligation of four of these primitive RNA molecules,[50] such that a new higher order force balance (and stable 3D form) was established.

Because a local force can produce global structural rearrangements and change the shape of an entire prestressed tensegrity structure without breaking any connections,[3,6] the binding of a protein or RNA to another molecule can cause their different stiffened microregions to rearrange their relative positions throughout the length of the molecule. Because of this flexibility, one molecule can change conformation to "fit" or tightly interlock with other; allosteric interactions also become possible. The resulting transfer of stresses between bound molecules triggers a cascade of molecular restructuring events that lead to new and critical functions, including catalysis and signal transduction, as well as pathological functions, such as prion self-replication. The following is a simple example of the power of molecular mechanics. Inorganic catalysts and organic enzymes often exhibit similar catalytic mechanisms, however, the enzyme exhibits greater structural *flexibility* such that the reactants and products come on and off the activated enzyme complex with much less exchange of energy and reaction efficiency is therefore greatly increased.[31] Thus, once they emerged, these more flexible RNA- and peptide-based protoenzymes progressively replaced the more rigid clay minerals and inorganic catalysts that could not compete in terms of efficiency or versatility.

Proteins and RNAs produced by clay were likely small in size, simple in shape, and relatively random in sequence. If one views their continuous production by clay as a primitive combinatorial chemistry set-up, however, then it would be surprising if some self-replicating molecules did not develop. The ability of modern in vitro selection approaches to rapidly create short peptides[51] and RNA-based enzymes ("ribozymes"; Ref. 52) with this self-replicating capability makes this point directly. Yet, virtually no molecule autonomously catalyzes its own formation in living cells. Self-replication may have been selected out during evolution because it interferes with progressive self-assembly and selects for itself only. Probably more relevant is the creation of cross-catalytic networks in which different molecular elements catalyze each other's production.[53,54] Remnants of self-replicating RNA molecules that rely on cross-catalytic contributions may be found in the Group I introns of modern genes[55] and in plant viroids.[56]

One of the simplest and yet most important functions necessary for the evolution of larger functional molecules may have been the ability to catalyze the folding of other molecules. Today, we recognize "molecular chaperones" which catalyze protein folding and related heat-shock proteins that bind and inactivate denatured proteins.[57] These folding-modulators may be remnants of early steps in the evolutionary path that led to modern cells and, when assembled into filamentous scaffolds (e.g., as in certain Archaebacteria; Ref. 9), the forerunners of the eukaryotic cytoskeleton. Primitive heat-shock proteins also may have helped to stabilize the evolving molecular complexes against high temperatures, oxidants, and heavy metals in the primordial earth environment.

Emergence of self-replicating multimolecular machines

A range of RNAs of different size, sequence, shape and function would come into existence due to the low fidelity of primitive autocatalytic ribozymes. Eventually RNAs would emerge that could ligate other RNAs, thereby resulting in

production of longer RNAs. As shown with group I introns,[56] complementary binding interactions between the loop regions of different RNAs would result in formation of base-paired structures with higher mechanical stability and thus, longer half-lives. Based on recent studies,[58,59] other ribozymes would appear that functioned like primitive aminoacyl tRNA synthetases and peptidyl transferases, which catalyze the formation of amide bonds between RNA and amino acids and between different amino acids. Through the action of these primitive amino acid synthetases, some of the bound RNAs would contain covalently linked amino acids. When two of these RNA–amino acid units, by chance, bound to adjacent base sequences of the same longer RNA (via the exposed nucleotides on their loop regions), then joining of the amino acids would be catalyzed by the peptidyl transferase ribozyme resulting in the production of the first peptides using entirely organic means. This was the birth of the primordial ribosome and the end of the era of clay. At this time, genes would not yet exist to code for sequence-specific information; nevertheless, the efficiency of the combinatorial chemistry set-up would increase dramatically when these primitive protein–RNA machines came into existence.

Here is where autocatalytic sets and solid-state biochemistry (Table 1) likely came into play. Ribozyme-produced peptides that bound back to the same molecular assemblage (i.e., containing the "coding" RNAs as well as ribozymes that catalyzed synthesis of proteins, RNAs, and RNA ligation) and mechanically stabilized this web of interactions would self-reinforce their own formation and lead to production of longer RNAs.[60] The longer the RNA, the longer the peptides that would be produced; the longer the proteins and RNAs, the greater the mechanical strength of the scaffold that would hold together the metabolic web of interactions. Furthermore, if chaperonin-type peptides were synthesized that formed filaments as in Archaebacteria,[9] this would both further strengthen the scaffold and increase protein-folding efficiency. The combination of solid-phase chemistry with chemical replication would also result in a huge (exponential) increase in the local concentration of products and hence potential reactants for downstream processing steps.[61]

Importantly, when proteins and RNAs were produced in excess by these protein synthetic machines, entirely new replica scaffolds would self-assemble and the process would renew. Hence, the first self-replicating multimolecular machines would emerge. But these primitive structures would still depend on their local microenvironment for other key elements necessary for their replication (e.g., molecular precursors, energy-rich molecules, etc.) and thus, they were probably only short-lived.

In modern cells, ribosomes similarly form continuously throughout the life of the cell through self-assembly of proteins and RNAs. The only difference is that the major structural rRNAs are now coded by DNA and produced in the nucleus before their transport to the cytoplasm where this vestigial form of self-assembly takes place. Centrosomes that nucleate the polymerization of microtubules in the mitotic spindle similarly must self-replicate during each cell cycle independently of DNA in order for complex eukaryotic cells to divide.[62] In certain organisms (e.g., *Paramecium*), the submembranous cytoskeleton even displays structural inheritance.[63] RNAs that associate with cytoskeletal filaments[64] could play a role in these processes.[65] Thus, functional ribonucleoprotein complexes, such as ribosomes, may represent living microfossils that retain some of the scaffold-directed self-replicating mechanisms that mediated earlier phases of biological evolution.

Transfer of control to proteins and DNA

Examination of living cells reveals that proteins alone are sufficient to self-assemble together to form important cellular structures and chemical processing complexes (e.g., cytoskeletal networks, signal transduction complexes, viral capsids) whereas RNAs and DNAs almost never appear in the absence of bound proteins in modern cells. Based on the increased number of amino acids versus ribonucleotides, proteins also display more diverse structures and functions. Thus, if cellular morphogenesis mimics cellular biogenesis, then, once more efficient protein production capabilities emerged, proteins would be expected to take over many of the roles that primitive RNAs once carried out. This is the beginning of the world in which we now live.

Effective RNA-dependent DNA polymerases (reverse transcriptases) would likely emerge only after production of larger proteins became possible; the first long DNA molecules would then enter the scene. Some of these DNA polymerases may have synthesized double-stranded DNA from nucleotide precursors without a primer–template complex, as observed in Archaebacteria.[66] Interestingly, the repetitive DNA sequence that is produced by these Archaebacterial enzymes is similar to that found in centromeric satellite DNA of modern organisms; this may be another living microfossil.

Through relatively minor structural modifications, DNA-directed RNA polymerases would also come into existence. If DNA molecules that were synthesized from RNAs bound back to the same coding RNA–protein scaffolds, and if these scaffolds contained this new type of RNA polymerase, then new higher order autocatalytic webs would arise that would self-reinforce production of the same RNAs and proteins from DNA. The propensity of single strands of DNA to form mechanically strong double helices would feed back to further stabilize these solid-state catalytic scaffolds. The immobilization of the ends of different DNAs in close proximity also would increase their chance for ligation, resulting in creation of longer and longer DNAs. It is not difficult to imagine the emergence of novel polymerases that could now use two single strands of an opened DNA helix to direct the production of replica helices,

rather than RNAs; hence, DNA self-replication would come to life. Thus, the modern day genetic code would eventually emerge as a later improvement on the original codon–anticodon binding interactions of the primitive RNAs through reverse engineering.[44,45,50]

If the RNAs that directed the production of the first DNAs coded for proteins that were selected because they stabilized the primitive ribosome, then these DNAs would exhibit sequence specificity that had biological relevance: they would code for complementary RNAs that would direct the synthesis of these very same proteins and, hence, ensure self-assembly of an equally functional protein synthetic machinery. Large protein scaffolds also would be required to hold the long DNAs in place while other immobilized motor proteins could unfold and unwind the stiff molecule (e.g., helicases, topoisomerases). Thus, transcription machines that integrated into primitive ribosome scaffolds likely would be selected out over time. DNA molecules, which exhibit much greater stability (longer half-lives) than RNAs, would support production of many more copies of the same RNAs (and proteins) and with greater fidelity than ever possible previously, thereby reinforcing the stability of the coupled transcription–translation complexes. Importantly, elements of this very type of hierarchical structure, i.e., containing specialized subassemblies that mediate transcription and translation using solid-state biochemistry, may be found in modern bacteria.[10]

The formation of large protein–RNA scaffolds containing long DNAs that could reversibly flex between double- and single-stranded forms in local regions (i.e., primitive chromatin) would therefore open an entirely new path for evolution: access to DNA as the ultimate information storage molecule. The eukaryotic cell nucleolus, which contains both proteins and the genes (DNA/RNA) that encode rRNAs forming the backbone of ribosomes, incorporates many of these features. Interestingly, this paradigm offers a testable hypothesis: comparative analysis of the 3D structure of ribosomal proteins and RNAs should be more indicative of the earliest events in cellular evolution than analysis of rRNA sequences which are generally the focus of current evolutionary studies.

Structural diversification

As the length of proteins increased, the number of new functions would likely rise in parallel. Synthetic cascades involving groups of enzymes would develop to produce larger and larger biomolecules as well as mechanisms to cleave them into basic building blocks that could feed back into the synthetic cascade. Coupled transcription–translation scaffolds that incorporated enzymes (and their coding DNA and RNA) with novel functions would lead to new autocatalytic webs and, thus, the first specialized elements of cellular metabolism would come to life. Soon proteins that could bind to themselves and form stable 3D scaffolds in the absence of RNA came into existence; these would represent the first attempts at polymerization and depolymerization displayed by modern cytoskeletal filaments. Preferential binding of RNAs to distinct topological features on specific scaffolds may have provided an early selection mechanism to increase the fidelity of subcomponent reproduction within each particular type of functional module. Remnants of this type of modularity can be seen in eukaryotic cells in which specific mRNAs localize on the cytoskeleton in microdomains where their protein products distribute.[64,67]

In summary, the immobilization of protein enzymes, RNAs and DNA on rudimentary cytoskeletal scaffolds served to bring substrates and reactants into close proximity and, thus, to accelerate further the formation of autocatalytic webs of chemical reactions, including additional self-assembly reactions. These scaffolds provided a physical basis to interlink different chemical processes and to pass energy through the whole system. When proteins bound to stable multimolecular scaffolds, motive forces generated through elastic conformational changes in the molecules also became apparent. The first molecular motors—progenitors of myosins, dyneins, and kinesins—soon emerged. Once one filament could be pulled along a neighbor by molecular motors, contractility, movement, shape changes, and the ability to ''engulf'' other large structures all become possible. The existence of different structural scaffolds with specialized solid-state catalytic functions (e.g., glycolysis, catabolism, anabolism, energy production, proton transport, contractility) that could be moved en masse as stable integrated modules were likely critical for their later self-assembly and consolidation into a single cell. This type of scaffold-based integration between structure and function that involves coordination of many smaller functional modules is observed today in both eukaryotes and prokaryotes.[2,9,10,12,14,19,20,23,24,25,31,68]

Development of the cell membrane

Long-chained lipids, which can form spontaneously under pre-biotic conditions,[69] self-assemble into membrane-lined sheets and vesicles driven by the need to minimize energy. However, like RNA and DNA, pure lipid bilayers rarely appear in the absence of proteins inside living cells; membranes almost always appear tightly coupled to underlying protein cages (e.g., viruses) or to more complex cytoskeletal structures. Because of energetic issues, it has been suggested that primitive membrane scaffolds evolved first from self-assembly of amphipathic proteins and that a second self-assembly event later joined them together with phospholipids, which then filled in the spaces.[70] In fact, this how new membrane vesicles form in living cells during endocytosis. Clathrin proteins self-assemble into large arrays of hexagonal and pentagonal forms that create geodesic domes and spheres directly beneath the surface membrane. Phospholipids in the surface membrane then join with these geodesic networks to form membrane vesicles that pinch off from the membrane and move

throughout the cell. This process may be a remnant of the past when the first differentially permeable membranes first came into existence. This endocytic mechanism also could have led to engulfment of other molecular assemblages prior to the evolution of a more complex contractile apparatus. Thus, one possibility is that evolving transcription–translation machines that expressed appropriate proteins on their outer surface might have pulled lipids out from the surrounding environment, thereby spontaneously forming a surface membrane enclosing their catalytic machinery. This latter hypothesis is supported by recent evidence that suggests that the transcription–translation machinery of bacteria—the closest living relative of the original progenitor cell—is also coupled to the membrane protein insertion machinery.[10,71]

The first cells

The most primitive cells likely exhibited specialized metabolic webs and only functioned as "living cells" when in microenvironments that provided the missing functions, much as viruses are only "living" when they infect cells. However, progressive coalescence of primitive membrane-lined cells that contained self-renewing, transcription–translation scaffolds with other cells containing similar multimolecular machines with different functions (e.g., production of energy-rich ATP molecules, synthesis of critical molecular building blocks, etc.) would eventually result in formation of cell containing a higher order autocatalytic web that would again self-reinforce its own formation. Those cells that exhibited the greatest ability to engulf other functional translation–transcription complexes would be the most likely to diversify operations and, hence, to become autonomous. These cells also would be the most likely to take in new machinery for production of contractile elements that would further accelerate the process of engulfment and structural diversification. The progressive joining of multiple transcription–translation machines, each with different functions encoded by its own DNA, is consistent with development of the well-known modular structure of the modern genome.

The stability of these primitive membrane-lined cells would be enhanced by the production of membrane proteins that could function as transmembrane ion channels. This would allow cells to control osmolarity and pH and, thus, prevent the membrane disruption. Because the lipid bilayer is more elastic than submembranous protein scaffolds, those ion channels that maintained physical connections to the skeletal backbone of the transcription–translation–membrane insertion machinery might be the most well adapted to sense mechanical stresses in the environment (e.g., osmotic swelling). These cells would be able to respond by altering ion transport across the membrane or by inducing compensatory changes in transcription, translation or membrane protein insertion. These would be the progenitors of today's ubiquitous stretch-sensitive ion channels and signal-transducing receptors, which similarly can be activated by applying mechanical stress to surface proteins that link to the internal cytoskeleton.[3,4,23,24,72] Interestingly, application of stress to these transmembrane linker proteins in eukaryotic cells induces recruitment of ribosomes to the cytoskeleton directly beneath the local site of force application on the cell membrane.[23]

Recent work on bacteria suggests that the primitive membrane-lined cells with the most DNA may have been preferentially selected because of DNA's mechanical ability to prestress and hence stiffen the surface membrane against potentially disruptive osmotic forces,[71] another example of shape stabilization through tensegrity. This would lead to selection of cells with increasingly long DNAs which, in turn, would encode for increasing numbers and types of molecular components. Stable L-type bacteria that can survive without their cell wall by overexpressing DNA[73] and nuclei of eukaryotic cells, which contain DNA wound up into coils and compressed within a tensed RNA–protein lattice surrounded by a protein–lipid membrane,[4,14,20] might not look that different from these primitive cells. DNA even appears to form a single long, mechanically continuous thread in eukaryotic cells that ostensibly display isolated chromosomes during mitosis.[74] Thus, the majority of DNA, which is currently thought of as "junk", may play a critical structural role by orienting critical catalytic machinery and mechanically stabilizing higher order structure in living nuclei[14,20] as it did in primitive prokaryotic cells.

Once cells developed the ability to provide themselves with energy and building blocks necessary continuously to fuel the growth and function of their autocatalytic webs and orienting scaffolds, they would become self-perpetuating. However, while elements of the subcellular machinery (e.g., primitive ribosomes) could reproduce or expand in number, the whole cell could not. The cells might expand their functions by salvaging remnants (self-stabilized subcomponents) of other cells and by random mutation; however, diversification would proceed slowly. Finally, one or more of these cells developed a mechanism to coordinate the complete separation of all of the cell's self-replicated scaffolds, their attached metabolic machinery, and their surrounding membranes such that one cell could now divide into two. This apparently involved the emergence of primitive cytoskeletal proteins that can self-assemble into contractile (tensile) rings that pinch one cell into two like a purse-string. The descendants of these proteins are the Fts-Z of bacteria and Fts-A of mitochondria and chloroplasts. These proteins resemble the tubulin molecule, which self-assembles into microtubules that form the mitotic spindle of eukaryotic cells.[11] In this manner, cellular life was created: the first true autonomous self-reproducing cells entered the stage and, as they say, all the rest is history. [See Ref. 41 for a description of how eukaryotic cells similarly arose from self-assembly (symbiosis) of these primitive life forms.]

Conclusion

Biologists tend to discuss life at the cellular level in terms of chemistry, molecular binding interactions and gene expression. The essence of my story is that life is structure in its most miraculous form. However, it is not simply the structure of DNA or of any other single component, that is of greatest importance, but the structure of the entire living hierarchy and the rules that guide its self-assembly.

Evolutionary biology is currently focused on how genetic information is worked upon by natural selection. In contrast, I presented plausible scenario (Table 2) for how cellular life may have emerged as an inevitable result of Nature's design process at work and the need to satisfy basic microarchitectural and energetic constraints. Because of these simple design requirements and the high efficiency of tensile structures, Nature selected tensegrity as the most economical solution to ensure shape stability at all size scales. These are the stable structures that also provide the flexibility necessary to self-assemble with other structures, to catalyze molecular modifications, and, in general, to explore potential evolutionary space.

The emergence of DNA and genes late in this process gave rise to a new mechanism for generating structural diversity that accelerated evolution and led to the creation of cellular life as we know it today. Yet, throughout all history, the design rules guiding the process of hierarchical self-assembly remained essentially unchanged. In retrospect, D'Arcy Thomson, in his brilliance, must have sensed this when he suggested that patterns in Nature represent "diagrams of underlying forces" and when he tried to explain how different species emerged through progressive mechanical distortion of a basic body plan.[16] Unfortunately, he lived at a time when he was limited to comparative study of macroscopic forms, rather than of cellular architecture on the microscale; so he never was able to appreciate fully the intricacy of this process. Many others remain blind to the importance of microarchitecture for evolution because they continue to view the cell as a membrane sac filled with viscous fluid containing DNA, metabolic cascades and signaling molecules. It is only when the microstructural complexity of the cell and the solid-state nature of its metabolism are taken into account that all the puzzle pieces fall into place. So the answer to the question of how life evolved on this planet may have been right there in front of us all the time. It was merely hidden in the microscopic structures of current living cells and in the processes that guide their hierarchical self-assembly.

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