

PUNTI DI VISTA / VIEWPOINTS

The Fractal Revolution: To See a World in a Grain of Sand

Giuseppe Damiani

*To see a world in a grain of sand
And a heaven in a wild flower
Hold infinity in the palm of your hand
And eternity in an hour.*

William Blake – *Auguries of Innocence*

The genesis of form in Nature is an important issue which gives rise to many controversial explanations. Some of these dichotomies regard the methodological aspects, others the substance of different theories. The evaluation of these antithetic explanations could be very useful to highlight some different aspects of the problem. The philosophers and scientists of the ancient time have developed many different cosmogonies and mythologies to explain the origin and the transformation of natural forms. The Greek philosophers Plato and Aristotle give an important contribution to the development of the occidental culture. For Plato the Universe is basically simple and the forms of natural objects are the results of geometric invariant archetypes. For Aristotle the world is a complicated series of changing things. During the 20th century, the development of human thought was dominated by science and technology. The aim of scientists is to discover and to explain regularities in patterns of natural phenomena. These regularities can be described by natural laws that are expressed in mathematical terms: “The book of Nature is written with mathematical symbols” (Galileo Galilei).

For Dirac and many other scientists the “beauty” and “elegance” of a mathematical model are important guides for the formulation of correct theories of Nature. Euclidean geometry and analytical calculus, typically in the form of linear differential equations, are the standard way of modelling the laws of nature. These mathematical tools allowed the formulation of laws describing simple phenomena with repeatable behaviour. Today’s best working descriptions of the ultimate natural laws (the so-called “standard model”) are based on gauge symmetries. The discoveries of the modern physicists confirm the Platonic assumption that invariant geometric blueprints are the fundamental cause of natural forms. But, also for Plato, there is a difference between the irregularities of the real objects and the regular unchanging blueprints that governed them. Our world is full of complex systems with an irregular and unpredictable behaviour. The traditional development of science was unable to find a unitary explanation for both simple and complex systems. With the increasing amount of human and financial resources devoted to the scientific research the science has been fragmented into many different specializations. The processes of specialization and the reductionistic approaches have an operative meaning, because the concentration of attention and efforts about only one object and problem may immediately produce applied results. But the specialization of science has also negative consequences. Some fundamental aspects of the reality can be disregarded. A specialised approach goes against our belief in the unity of Nature and is unable to solve some important problems that require interdisciplinary and systemic approaches. It is important to consider not only the isolated objects, but also the network of relations existing among them. The investigation of complexity of natural systems requires new mathematical approaches. The development of the “fractal geometry” is the main advances for the scientific and unitary understanding of complex systems.

Benoit Mandelbrot (20 November 1924 – 14 October 2010), known as “father of fractal revolution”, has died this year at age 85. Mandelbrot “conceived and developed a new geometry of nature and implemented its use in a number of diverse fields” (Mandelbrot [1975]). He coined the term *fractals* to describe “many of the irregular and fragmented patterns around us”. Linear

equations and traditional Euclidean geometry represent a powerful abstraction of reality, but for understanding the intimate nature of the reality, they turn out to be a wrong kind of abstractions. "Clouds are not spheres. Mountains are not cones. Lightning does not travel in a straight line". Linear studies are popular because the solutions are easy to obtain. With the coming of computers, Mandelbrot developed a new kind of mathematics which deals with discrete entities rather than continuous sets of points and with approximate numerical solutions of both linear and non linear equations rather than exact analytical solutions of linear equations. In particular, Mandelbrot developed the "fractal geometry" that describes irregular shapes with similar properties at different scales. The mathematical community debates the value of these new discrete models that are mainly deduced through computer simulations instead of a series of theorems and proofs. Some traditional mathematicians believe fractals are just pretty pictures. Mandelbrot replies that "pretty pictures in the appropriate minds lead to pretty problems and entire new fields". Undoubtedly computer simulation and fractal geometry are very useful tools to understand structures and dynamics of complex systems generated by simple iterative rules. A wide range of natural structures has been quantitatively characterised using the idea of a fractal dimension. In particular many biological structures have fractal, branching and vascular morphologies: trees, venation of leaves, lungs and in general most circulatory and transport systems in living organisms, suture lines of ammonoids, aggregated structures produced by myxobacteria and myxomyceta, chromatophores of some animals, nervous cells and many others (Figure 1). Computer simulations based on fractal geometry and non-linear equations allow realistic reproductions of patterns and processes characterising natural systems. Similar physical laws and forces acting in the same way at the different levels produce structures with self-similarity at different levels.

When I was eight years old, during an excursion to the mountains of the central Apennines, I found a small fossil shell of ammonite. I was surprised by this discovery and by the beautiful vascular patterns of the suture lines on the shell. At first, I thought that these patterns were the imprints of seaweed, but my father said to me that this idea was wrong. Then some questions arose in

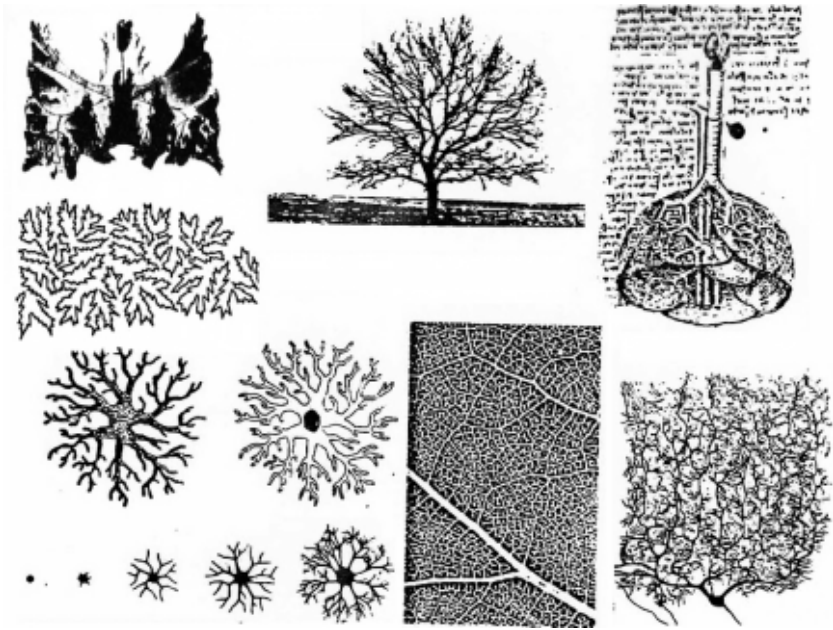


Fig. 1 – Biological structures with fractal shapes: an ammonoid septa, a tree, a pulmonary tree, an ammonoid suture line, some cromatophores, a leaf, and a Purkinje cell.

my mind: Why the suture lines morphology is analogous to that of seaweed? What is the functional meaning of this complex structure? Why the natural objects have the form that they have? In 1979 I have written a work on the origin, function and fabrication of fluted cephalopod septa. These structures have a fractal form and have had the tendency to increase their complexity during the ontogenetic and phylogenetic developments. The analysis of the mechanical principles involved in the static of the cephalopod shell leads to the conclusion that the trend to increasing complexity is useful to maximise the shell volume and resistance to hydrostatic pressure minimising, in the same time, the shell weight. As Galileo Galilei wrote “it would be a good thing to know the optimal form of a structure to avoid that a weight loaded on a point of the structure could break it more easily than if it was loaded on another

point". I realised that analogous optimisation problems are very common in Nature. Are symmetry and regularity of natural forms and patterns the results of fractal processes? Are there simple universal laws that allow us to comprehend them? For D'Arcy W. Thompson [1917] the forms of living organisms are determined mainly by simple physical laws: "forms are diagrams of forces". Natural systems show a great diversity of complex patterns and behaviours. Very simple component parts, organised according to simple iterative laws, may generate this complexity. I have hypothesised that the enormous variety of the complex fractal morphologies of the cephalopod septa and of many other branching structures is produced by few simple iterative rules based on the reaction-diffusion model. In 1952, Alan Turing suggested that diffusion and reaction of few entities produce the morphogenetic development of several chemical and biological systems. According to Turing, a reaction-diffusion model may explain the ontogenetic and phylogenetic upward trend of complexity of many biological branching structures. The function of these structures in living organisms is to distribute or to gather biological material and physical entities. Optimisation processes produced by natural selection led to minimisation of the networks lengths (the Steiner problem) and of the amount of information needed for their construction. What is the optimisation method chosen by nature? Experimental data and computer simulations suggest a simple way to generate minimised network: sensitive entities spread or concentrate in a surface or in a volume responding to morphogenetic gradients of physical forces or of chemical substances. Recursive local rules of repulsion or attraction lead to the formation of fractal, branching, vascular and global networks (Figure 2). Slight changes of morphogenetic fields and of responsiveness of sensitive entities influence the shapes of produced patterns. Natural selection allows the survival of the biological structures most suitable for their physiological functions: to distribute or to gather something in an economic (cost function minimisation), uniform (space-filling) and size-independent (self-similar scaling) way. Computer simulations by means of genetic algorithms based on the same optimisation method chosen by nature may be applied successfully to solve a wide variety of scientific and engineering problems (Damiani *et al.* [1997]).

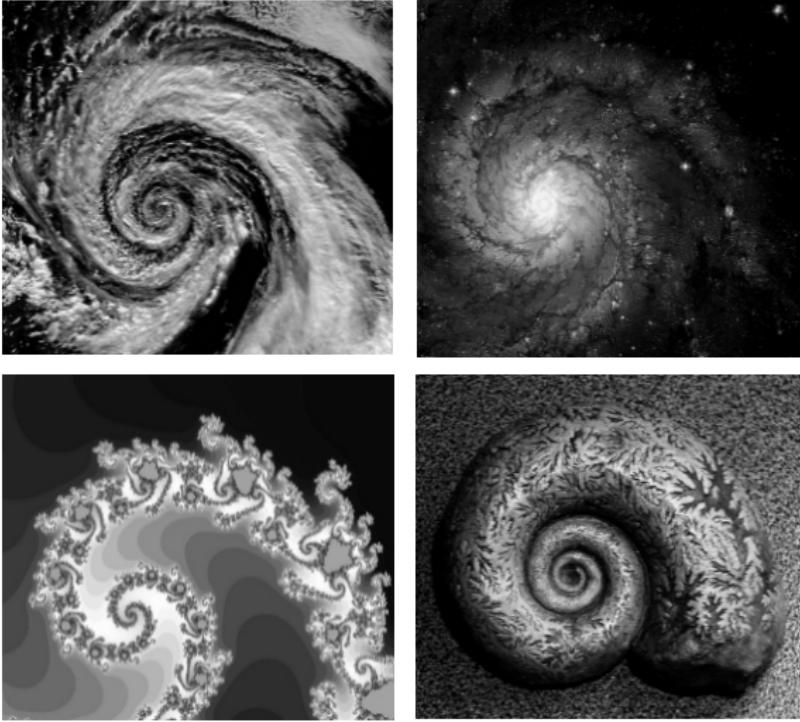


Fig. 2 – Fractal spirals of different size: the galaxy M74, a cyclone, a pattern from Mandelbrot set, and an ammonite.

The recent developments in different interdisciplinary scientific areas (fractal geometry, information theory, analysis of complex and dissipative systems, emergence of auto-organization, chaos theory, and cellular automata) are creating new ways of thinking about Nature. These studies have revealed that chaotic behaviour may be the results of the non-linear interactions between the different elements of a complex system. Chaotic behaviour is produced by sensitivity to initial conditions: trajectories that start very close together diverge rapidly. Even if a system is controlled by a set of completely deterministic rules, its behaviour may be unpredictable because real measurements are not precise enough to distinguish nearby trajectories that may later diverge. Surprisingly, as

discovered by Feigenbaum, the chaotic behaviour has a fractal form: many different systems show the same universal properties in the scenario of trajectories bifurcations. Why the transition to chaos has a fractal shape? The study of complex systems has shown that simple interactions sometimes lead to very complicated structures and behaviours. “Conversely, complex interactions can lead to simple results. This is particularly true for systems formed of many interacting subunits, such as liquids, solids or gases, which are poised at a ‘critical point’ where two or more macroscopic phases become indistinguishable. At a critical point, many of the precise details of the interactions between constituent subunits play virtually no role whatsoever in determining the bulk properties of the system. At T_c (critical temperature) the rapid exponential decay turns into a long-range power-law decay of the form $C(r) = r^{-n}$ where $C(r)$ is the correlation between subunit separated by a distance r , and n is called a critical exponent. If correlations decay with a power-law form, we say the system is ‘scale free’, because there is no characteristic scale associated with a simple power law... At one time, it was imagined that the ‘scale-free’ case was relevant to only a fairly narrow slice of physical phenomena. However, the range of systems that apparently display power-law correlation has increased dramatically in recent years, ranging from base-pair correlations in DNA, lung inflation and interbeat intervals of the human heart, to complex systems involving large numbers of interacting subunits that display ‘free will’, such as city growths and even economics. The principle of universality seems to be reflected in the empirical fact that these quite different systems can have remarkably similar critical exponents – perhaps because the ‘interaction paths’ between the constituent subunits in such extremely complex systems dominate the observed cooperative behaviour more than the detailed properties of the subunits themselves” (Stanley *et al.* [1996]). This long citation of Stanley explains in a clear way one the most universal and mysterious law underlying the form of each transformation happening in our Universe: each phase transition, at its critical point, has a fractal pattern. The characteristic behaviour of real physical systems is a mix of regularity and irregularity, of order and disorder. This is true at all levels, from the micro-world of quantum mechanics to the macro-world of cosmology.

Fractal-chaos, at the border of the phase transition between order and chaos, is everywhere.

At the molecular level, the evolutionary dynamics of chemical systems are the result of the competition between catabolic reactions, which degrade energy and chemical compounds, and anabolic reactions, which increase the complexity and the configurational order of material structures. In this scenario, the evolution of life on earth is not a fortuitous event, but is the unavoidable result of the constant flux of solar energy in a large system of molecules, which are able to perform cyclic reactions. Biological systems might be produced by an auto-regulatory feedback loop between opposite autocatalytic activities of catabolic and anabolic types. This mechanism, named metabolic hypercycle, is described by the Lotka-Volterra prey-predator equation and gives rise to fractal spatial-temporal patterns and rhythms (Damiani [2007]). According to Zipf's principle of the least effort, the dynamic stability of the critical states of complex systems is produced by the minimisation of distributions and flows of opposite and correlated processes. Maps and clocks based on metabolic hypercycles might be used by living organisms to maintain their homodynamic equilibrium and biodiversity at different levels. The understanding of the dynamics of complex biological systems is very important to show the limits of the neo-Darwinian synthesis and to highlight the mechanisms of many physiological, evolutionary and ecological processes. The availability of a unitary theoretical framework for a rational interpretation of many physiological data may help the scientific community to develop effective treatments of unbalanced biological systems. In particular the understanding of the functioning of the neuroendocrine-immune network will lead to the development of integrated therapies of complex diseases such as many metabolic, neuropsychiatric, and autoimmune disorders, which are still poorly understood.

Istituto di Genetica Molecolare, CNR, via Abbiategrasso 207, 27100 Pavia (Italy)
E-mail: damiani@igm.cnr.it

REFERENCES

- Damiani, G. and P. Della Franca [1997], Morphé and Evolution. *Riv. Biol./B. Forum* **90**: 227-266.
- Damiani, G. [2007], The Yin and Yang of Anti-Darwinian Epigenetics and Darwinian Genetics. *Riv. Biol./B. Forum* **100**: 361-402.
- Mandelbrot, B. [1975], *Les objets fractals: forme, hasard et dimension*. Flammarion, Paris.
- Stanley, H.E., L.A.N. Amaral, S.V. Buldyrev, A.L. Goldberger, S. Halvin, H. Leschhorn, P. Maass, H.A. Makse, C.-K. Peng, M.A. Salinger, M.H.R. Stanley, G.M. Viswanathan, [1996], Scaling and Universality in Animate and Inanimate Systems. *Physica A* **231**: 20-48.
- Thompson D'Arcy, W. [1917], *On Growth and Form*. Cambridge U.P., Cambridge.
- Turing, A.M. [1952], The Chemical Basis of Morphogenesis, *Philosophical Transactions of the Royal Society* **B237**: 37-72.

Quiescence and Stress: A Bioelectrical Viewpoint

Silvano Traverso

1. *Does Polymerization/Depolymerization of the Cytoskeleton Correlate with Quiescence/Activation of the Cell?*

The cytoskeleton, the cellular meshwork made of filamentous actin, microtubules and intermediate filaments, is a dynamical, continuously rearranging structure. Both actin and microtubules undergo continuous polymerization and depolymerization and such dynamics seems to have a fundamental physiological significance. Indeed, a high actin dynamics seems to be a necessary condition for the well-being of the cell: if the dynamics slows down, large ADP-actin aggregates form and a massive production of Reactive Oxygen Species occurs, which eventually leads to apoptosis (Gourlay and Ayscough [2005]).

The cytoskeleton dynamics is the result of two opposing tendencies (namely polymerization and depolymerization). This would likely lead to *chaotic* dynamics, as occurs in many natural processes in which opposite forces interact (Damiani and Della Franca [1997]; Aon *et al.* [2000]). For instance, at a certain threshold (e.g., a critical tubulin concentration) microtubules show a “dynamic instability” between assembly and disassembly, with occasional dramatic depolymerization events (called “catastrophes”) followed by polymerization (“rescues”), which occur in an apparently random way. This behaviour is typical of the so-called *self-organized criticality*. Perhaps, rather than random, the microtubule dynamics should be more appropriately regarded as chaotic.

Staying on the fractal border of a phase transition would make the cytoskeleton *optimized* (Aon *et al.* [2004], Traverso [2005]), allowing it to respond, by either polymerizing or depolymerizing, to minimal cues. So, dynamic instability would facilitate cytoskeletal fast responses (somewhat in the same way as the unstable equilibrium of the erect stance makes us ready to move in any direction).

When the cell is quiescent, the dynamics is slow and the cytoskeleton appears as a dense polymer network, which increases

the stiffness of the cell. Cell contraction is not allowed, nor is mechanotransduction (which requires cytoskeletal rearrangements). Diffusion is hampered. The nucleoskeleton, which is in mechanical continuity with the cytoskeleton, traps the chromatin preventing it from expressing its genes. The whole biochemical machinery works at its minimum.

When a stressing stimulus (of whatever nature) activates the cell, prompt *depolymerization occurs*. Actin reorganization is a fundamental feature of the *early* response of the cell to extracellular stimuli (Papakonstanti and Stournaras [2008]).

In unicellular organisms depolymerization initiates the gel/sol transitions necessary for motility, which is a basic biological reaction to stress. Following a stimulus, an increase in intracellular calcium concentration is observed, in unicellular as well as in multicellular organisms. If a certain threshold is reached, calcium activates, among other proteins, gelsolin, which depolymerizes the actin cytoskeleton.

Another universal response to stress stimuli is gene activation. The transition towards a sol state (or, better, to a softer gel state) releases the mechanical constraints on chromatin, a fundamental step in gene activation.

Activating stimuli initiate enzymatic cascades that ultimately lead to an encounter between transcription factors and genes. The cytoskeleton plays a pivotal role in such enzymatic pathways. It has been stressed (Damiani [2005]) that most of the anabolic cascades (such as the Notch pathway) activate the glycogen synthase kinase-3 (GSK3) which phosphorylates hundreds of proteins involved in cell stasis and apoptotic death, whereas most of the opposite catabolic cascades (such as the Wnt pathway) activate the casein kinase-2 (CK2) which phosphorylates hundreds of proteins involved in cell activation (survival and proliferation). Very significantly, both GSK3 (Sun *et al.* [2009]) and CK2 (Canton and Litchfield [2006]) are important regulators of actin and tubulin cytoskeletons.

Cytoskeleton depolymerization is a crucial step in the early enzymatic response to stress. For instance, a variety of agents and conditions that depolymerize microtubules activate the sequence-specific transcription factor NF-kappaB and induce NF-kappaB-

dependent gene expression (Rosette and Karin [1995]). NF-kappaB is a protein (widespread within the animal kingdom, from cnidarians to mammals) which is modulated by CK2 (Brown *et al.* [2010]) and is involved in the rapid response to stress stimuli¹ (oxidative stress, cytokines, UV irradiation etc.).

In addition to the rapid depolymerization, a second strategy the cell seems to adopt to respond to noxious signals is a delayed polymerization (for instance with formation of the so-called actin “stress fibers”). *The cell stiffens as a reaction to the stimulus.*

Depolymerization and polymerization may be part of the same response. During cell activation, often polymerization follows the fast depolymerizing response. For instance, polymerization of the actin cytoskeleton is known to characterize activated B cells. However, rapid (15-30 sec.) actin depolymerization upon stimulation of the B cell receptor has been reported, followed by polymerization (Hao and August [2005]). It has been shown that such a depolymerization enhances the signals from the B cell receptor².

Just another example: Actin is phosphorylated in a PAK1-dependent manner in the early response to opioids, such response being characterized by filament depolymerization. In a later phase of the response, actin re-polymerizes forming new stress fibers (Papakonstanti and Stournaras [2008]).

Therefore, a strong correlation seems to exist between the biochemical state of a cell and its biomechanical state (which essentially depends on the polymerization of its cytoskeleton). In the following, the possibility will be explored that the quiescent or activated state of a cell also correlates with its bioelectrical state.

¹ Many tumours show a constitutive expression of CK2 and NF-kB, which sustains cell proliferation.

² Interestingly, however, while a partial disassembly activates the pathway, disrupting F-actin blocks the signalling. This is consistent with the hypothesis, put forward in previous papers (Traverso [2005], [2006], [2008]), that a loosely interconnected cytoskeletal network (soft-gel state) is optimized for the signalling within the cell, whereas either a dense network (stiff-gel state) or a disrupted network (sol state) do not allow signal transmission, and characterize pathological conditions (both a stiff gel state with an exceedingly slow actin dynamics and a sol state, where the cytoskeleton has completely disassembled, lead to apoptosis).

2. Capacitance/Conduction and Quiescence/Activation

It is known that the cell membrane behaves as a capacitor, due to the difference of electrochemical potential between outside and inside the cell. A capacitor is a circuit element in which two conductors are separated by a dielectric (insulating) material, so that a potential difference is established across the dielectric, and electric charges, being unable to cross the insulator, accumulate at its sides. The main characteristic of a capacitor is to store energy in the form of electric charges.

Nerve transmission through action potentials, and ATP synthesis are well-known examples of processes which use the energy previously stored capacitively in the membrane and then released due to a breakdown of the dielectric barrier (opening of channels) and transformed into biochemical work.

However, membrane potentials are not the only endogenous electric fields. Many electrochemical potentials are present in the body. Examples include transepithelial potentials (in the mV range) in glands and embryos, and transendothelial extracellular potentials in blood vessels. Biological tissues, thus, exhibit dielectric (capacitive) properties.

On the other hand, everybody has heard speaking of biological tissues as “electrolytic conductors”. As a matter of fact, biological tissues may be considered as *either conductors or dielectrics*.

The hypothesis here put forward is the following:

Biological systems are capable of shifting their electrical properties towards capacitance or conductivity depending on the functional needs, capacitance characterizing the quiescent/anabolic state, while conductivity being correlated with cell activation, and particularly with the stress response.

As will be discussed below, the shift between capacitive and conductive states is expected to crucially depend on polymerization dynamics.

Before discussing further the issue, two points should be stressed:

1. The concept of “quiescence” does not strictly apply to biological systems, which are actually in a permanent quest for dynamical equilibrium. Although non-linear physics should be more

appropriately used for describing biological tissues, oversimplified and approximate descriptions, such as the one here proposed, may turn out to be helpful.

2. Within biological tissues, different forms of energy (thermal, mechanical, electrical, chemical) are converted into one another. As far as electromechanical phenomena are concerned, the tissue can be regarded as an electrical machine, consisting in a complex network of active – generators, actuators, amplifiers – and passive – inductors, resistors, capacitors – electrical components, a machine capable of converting, bi-directionally, mechanical and electrical energy. In the present paper, the attention will be limited to the mechanical modifications affecting the passive electrical behaviour of tissues, in a sort of “switching” between conductive and (almost-)non-conductive conditions. This variability can be seen as the modulation of a “conservative” function capable of “storing” energy in electrical form.

2.1 The Cellular Level

Polymerization and depolymerization, which characterize on the biochemical level the quiescent and the activated state, respectively, may have a counterpart in the bioelectrical properties of the cell.

If a cell is in its resting state, or in an anabolic phase, its cytoplasm will be filled up with polymers, especially cytoskeletal polymers. These are charged macromolecules which bind counter-ions at their surface. Counter-ions remain at a certain distance from the surface of the polymer (due to the so-called *exclusion layer*), so that the whole structure is considered by biophysicists as analogous to a capacitor.

Therefore, it can be inferred that, when in a quiescent (anabolic) state, the cell accumulates charges, thanks to the capacitance of the polymers densely packed within the cytoplasm.

In a quiescent cell the concentration of free, unbounded charges is expectedly low, also thanks to sequestering organelles (such is the case, for instance, of Ca^{++}).

Once an external stress is applied, depolymerization occurs, and the cytoplasm shifts from a gel towards an electrolytic solution (cytosol). Counter-ions are not bound anymore and become free

ions, increasing the global conductivity of the solution. Ions, such as calcium, are released into the cytoplasm from the sequestering sites, and *significantly* increase the concentration of free charges (suffice it to consider that in order for the actin-severing gelsolin to be activated, cytoplasmic calcium concentration must rise from nano- to micromolar values).

Under stressing conditions, hydrogen ions concentration changes as well. For instance, massive ROS production and DNA damages lead to activation of poly(ADP-ribose) polymerases (PARP) which, as a byproduct of their reactions, produce a great number of protons, with a fast fall in intracellular pH³.

Many other examples of ion release upon stress stimuli could be listed.

Due to the increase in free charge availability, the electric state of the cell dramatically changes. The conductance increases, and endogenous nanocurrents may arise (for instance from an activated cell to the adjacent one through a gap junction). It should be stressed that whenever cytoskeletal rearrangements, local gel/sol transitions and in general mechanical changes occur, flows of charges (currents) are expected to arise, due to the piezoelectric properties of F-actin and microtubules, to the streaming potentials linked to solvent circulations and so on. Such endogenous nanocurrents may affect many enzymatic activities, causing an “electrostretch” and the unfolding of enzymes (Panfoli *et al.* [2010]). Moreover the possibility should not be ruled out that biochemical response to stress includes reactions (such as deamidation) that lead to a general increase in the charged/uncharged ratio of amino acids in proteins, which would render them more sensitive to endogenous currents (Calzia *et al.* [2009]).

It should be also stressed that charges generate electrical forces in macromolecules that can be transduced into mechanical movements due to electric attraction/repulsion, piezoelectric deformations, electro-osmosis, etc.

³ PARP activation also involves a reduction in the ATP level which leads to inhibition of the linker proteins that physically couple the cell membrane and the cytoskeleton (Titushkin and Cho [2009]). Consequently mechanical changes occur, which, as stressed below, are accompanied by electrical phenomena.

To sum up, from a bioelectrical standpoint, *stress mobilizes charges capacitively stored by polymers, giving rise (mainly via depolymerization) to ionic currents capable of doing biochemical and biomechanical work.*

2.2 The Sovracellular Level

In tissues, endogenous electric fields can arise, for instance between interstitial liquid zones (conductive) separated by epithelial cells with tight junctions (highly resistive) (Grimnes and Martinsen [2008]).

The presence of many interfaces and barriers in biological tissues makes the occurrence of interfacial polarizations extremely likely. ‘Interfacial polarization’ is the electrical phenomenon by which a potential difference arises at an interface due to the accumulation of charges at the opposite sides of the barrier (capacitor).

For instance, a transepithelial potential (about 40 mV) physiologically exists, endogenous electric fields direct nerve growth in vivo and it is known that electrical fields naturally arise during embryo development (McCaig *et al.* [2009]).

Potential differences are expected to exist even between some organs (Grimnes and Martinsen [2008]) and closed DC circuits in the body, with blood vessels serving as electric cables, (e.g. vascular-interstitial closed circuits) have been suggested to exist (Nordenström [1983]).

The existence of a “bioconductive connectional system” involving intra and intercellular structures, namely, nuclear matrix, cytoskeleton, extracellular matrix, intercellular junctions and integrins, has been proposed: along the polymers, electrons, protons, photons, excitation and polarization waves, as well as mechanical signals can flow (Bistolfi [1990]).

Within body tissues, endogenous electric fields and microcurrents are then a likely occurrence.

When a tissue is injured or generally stressed, local changes in pH and redox state occur, changing the bioelectrical conditions. Noxious stimuli can dissipate existing electrochemical fields and give rise to ionic flows. An extreme example is the injury current: an injury dissipates the transepithelial potential, allowing ions to move according to their gradients. Such current, which flows out-

side the wound, seems to be important for the healing.

As mentioned above, tight junctions between epithelial cells constitute an electrical barrier across which potentials arise. It has been shown that depolymerization of actin filaments results in disruption of tight junctions and in barrier breakdown (Papakonstanti and Stournaras [2008]).

Thus, it is tempting to hypothesize that, under generally stressing conditions, *capacitively stored potential energy is transformed into microcurrents, as a part of the tissue response to the stimuli.*

In many pathological conditions, then, an increase in ionic flows is expected. For instance, many inflammatory bowel diseases are associated with a loss of intestinal epithelial barrier function, which is expectedly accompanied by dielectric loss (microcurrents).

2.3 Is the Shift between Capacitance and Conduction Regulated by the Autonomous Nervous System?

An intriguing possibility is that in complex animals *polarizations (capacitive properties) are enhanced by parasympathetic activity while charge flows (conductive properties) are enhanced by sympathetic activation.*

As is well-known, parasympathetic nervous system shifts the metabolism towards anabolism and accumulation of energy. This could have its electrical counterpart in the charging of tissues' capacitive structures.

On the other hand, sympathetic activity, which mobilizes the energy reservoirs and prompts the organism to movement, i.e. to an explosion of currents both in the nervous and in the muscular system, would have its electrical counterpart in a generalized electrical conduction. If this is the case, cytoskeletal depolymerization is expected to occur at the cellular level upon sympathetic activation. Indeed, it has been demonstrated that adrenergic agonists induce actin depolymerization in human airway smooth muscle cells by a cAMP-dependent signaling pathway (Hirshman *et al.* [2001]). Actin microfilament disassembly by agonists that increase intracellular levels of cAMP has been also demonstrated in fibroblasts (Lamb *et al.* [1988]) and neutrophils (Downey *et al.* [1991]).

It is also interesting to notice that the release of catecholamines from activated cells depends on actin depolymerization: in resting

cells exocytosis is prevented by a subplasmalemma actin mesh. Stimulation, for instance by neuroactive steroids, such as dehydroepiandrosterone, induces fast depolymerization of F-actin and filaments disassembly, thus allowing vesicles exocytosis (Charalampopoulos *et al.* [2006]).

Thus sympathetic-related factors seem to share the property of enhancing depolymerization, and therefore, as previously discussed, of shifting the cell electrical properties towards an increase of conductivity.

On the other hand, anabolic (parasympathetic) conditions are linked to increased stiffness of cells, which, on the electrical side, corresponds to high capacitance and high electrical resistance. The parasympathetic-driven increase in polymerization is expected to lead, as discussed earlier, to an increase in the capacitive properties of the tissue. At the same time, highly interconnected, stiff gels induced by the parasympathetic anabolic shift are known to exhibit higher electrical resistance. For instance, it has been experimentally demonstrated that in collagen and algal sulfated polysaccharide crosslinked films the crosslink decreases the electrical conductivity of the film (Figueiró *et al.* [2006]).

The rise of potential differences in tissues may be enhanced by parasympathetic activation. Indeed, the parasympathetic component of the autonomic system likely plays a fundamental role in the generation of the transepithelial potential: it has been experimentally found that exogenously applied acetylcholine increases the potential difference of the tracheal epithelium, whereas the perfusion of the mucosa with atropine (anticholinergic) or the cutting of the vagus nerve decrease the transepithelial potential (Tamaoki *et al.* [1996]).

3. Conclusions

To sum up, there are some grounds to consider the possibility that, both at the cell and the tissue level, in the quiescent/anabolic/parasympathetic state energy is capacitively stored whereas in the activated/catabolic/sympathetic state ionic currents are enhanced.

Although the idea that cell quiescence and activation correlate

with different bioelectrical states dates back to the pioneering studies of Szent-Györgyi, and a link between the parasympathetic/sympathetic activation and an electrical charge/discharge process was hypothesized in the 1930s by the controversial student Wilhelm Reich⁴, very few further efforts have been made in this direction, so that, to date, the physiological role of endogenous electric fields and currents in cells, tissues and organs still remains largely unexplored. Hopefully, future experimental and theoretical studies on the capacitive and conductive properties of cells and tissues will shed some light on this intriguing issue. A better understanding of bioelectrical phenomena can also lead to further developments of electrodiagnostic and electrotherapeutic techniques.

ACKNOWLEDGEMENTS

I am grateful to Dr. Giuseppe Damiani and to Dr. Michele Gallamini for their valuable suggestions.

REFERENCES

- Aon, M.A., S. Cortassa, D. Lloyd [2000], Chaotic Dynamics and Fractal Space in Biochemistry: Simplicity Underlies Complexity. *Cell Biol. Int.* **24**: 581-587.
- Aon, M.A., B. O'Rourke, S. Cortassa [2004], The Fractal Architecture of Cytoplasmic Organization: Scaling, Kinetics and Emergence in Metabolic Networks. *Mol. Cell Biochem.* **256-257**: 169-184.
- Bistolfi, F. [1990], The Bioconductive Connectional System: Radiobiologic and Therapeutic Implications. *Radiol. Med.* **80**: 203-206.
- Brown, M.S., O.T. Diallo, M. Hu, R. Ehsanian, X. Yang, P. Arun, H. Lu, V. Korman, G. Unger, K. Ahmed, C. Van Waes, Z. Chen [2010], CK2 Modulation of NF-kappaB, TP53, and the Malignant Phenotype in Head and Neck Cancer by Anti-CK2 Oligonucleotides In Vitro or In Vivo via Sub-50-nm Nanocapsules. *Clin. Cancer Res.* **16**: 2295-2307.
- Calzia, D., I. Panfoli, S. Ravera, E. Dazzi, S. Gandolfo, I.M. Pepe, L. Vergani, A.M. Morelli [2009], Structural Modification of Proteins by Direct Electric Current from Low Voltage. *J. Biochem. Mol. Toxicol.* **23**: 309-317.
- Canton, D.A., D.W. Litchfield [2006], The Shape of Things to Come: An Emerging Role for Protein Kinase CK2 in the Regulation of Cell Morphology and the Cytoskeleton. *Cell Signal.* **18**: 267-275.

⁴ The author thanks Dr. Giuseppe Damiani for highlighting the analogies with Szent Györgyi's and Reich's ideas.

- Charalampopoulos, I., E. Dermitzaki, L. Vardouli, C. Tsatsanis, C. Stournaras, A.N. Margioris, A. Gravanis [2006], Dehydroepiandrosterone Sulfate and Allopregnanolone Directly Stimulate Catecholamine Production via Induction of Tyrosine Hydroxylase and Secretion by Affecting Actin Polymerization. *Endocrinology* **146**: 3309-3318.
- Damiani, G. and P. Della Franca [1997], Morphé and Evolution. *Riv. Biol./B. Forum* **90**: 227-266.
- Damiani, G. [2005], Evolution and Regulation of Metabolic Networks. In G.A. Losa, D. Merlini, T.F. Nonnenmacher, E.R. Weibel (eds.), *Fractals in Biology and Medicine*, Birkhäuser Verlag, Basel-Boston-Berlin.
- Downey, G.P., E.L. Elson, B. Schwab, S.C. III Erzurum, S.K. Young, G.S. Worthen [1991], Biophysical Properties and Microfilament Assembly in Neutrophils: Modulation by Cyclic AMP. *J. Cell Biol.* **114**: 1179-1190.
- Figueiró, S.D., A.A.M. Macêdo, M.R.S. Melo, A.L.P. Freitas, R.A. Moreira, R.S. de Oliveira, J.C. Góes and A.S.B. Sombra [2006], On the Dielectric Behaviour of Collagen-algal Sulfated Polysaccharide Blends: Effect of Glutaraldehyde Cross-linking. *Biophysical Chemistry* **120**: 154-159.
- Gourlay, C.W. and K.R. Ayscough [2005], The Actin Cytoskeleton: A Key Regulator of Apoptosis and Ageing? *Nature Reviews / Molecular Cell Biology* **6**: 583-588.
- Grimnes, S. and O.G. Martinsen [2008], *Bioimpedance and Bioelectricity Basics*. Elsevier, Oxford.
- Hao, S. and A. August [2005], Actin Depolymerization Transduces the Strength of B-Cell Receptor Stimulation. *Mol. Biol. Cell* **16**: 2275-2284.
- Hirshman, C.A., D. Zhu, R.A. Panettieri, C.W. Emala [2001], Actin Depolymerization via the Beta-Adrenoceptor in Airway Smooth Muscle Cells: A Novel PKA-independent Pathway. *Am. J. Physiol. Cell Physiol.* **281**: C1468-C1476.
- Lamb, N.J., A. Fernandez, M.A. Conti, R. Adelstein, D.B. Glass, W.J. Welch, J.R. Feramisco [1988], Regulation of Actin Microfilament Integrity in Living Monomuscle Cells by the cAMP-dependent Protein Kinase and the Myosin Light Chain Kinase. *J. Cell Biol.* **106**: 1955-1971.
- McCaig, C.D., B. Song, A.M. Rajnicek [2009], Electrical Dimensions in Cell Science. *Journal of Cell Science* **122**: 4267-4276.
- Nordenström, B. [1983], *Biologically Closed Electric Circuits*. Nordic Medical Publications, Stockholm.
- Panfoli, I., S. Ravera, D. Calzia, A. Morelli [2010], Accelerated Removal of Deamidated Proteins and Endogenous Electric Fields: Possible Implications. *Gen. Physiol. Biophys.* **29**: 302-308.
- Papakonstanti, E.A., C. Stournaras [2008], Cell Responses Regulated by Early Reorganization of Actin Cytoskeleton. *FEBS Letters* **582**: 2120-2127.
- Rosette, C. and M. Karin [1995], Cytoskeletal Control of Gene Expression: Depolymerization of Microtubules Activates NF-kappa B. *J. Cell Biol.* **128**: 1111-1119.
- Sun, T., M. Rodriguez, L. Kim [2009], Glycogen Synthase Kinase 3 in the World of Cell Migration. *Dev. Growth Differ.* **51**: 735-742.
- Tamaoki, J., A. Chiyotani, E. Tagaya, H. Takemura, K. Konno [1996], Cholinergic

- Control of Rabbit Tracheal Transepithelial Difference In Vivo. *Eur. Respir. J.* **9**: 1632-1636.
- Traverso, S. [2005], Cytoskeleton as a Fractal Percolation Cluster: Some Biological Remarks. In G. Losa, D. Merlini, T. Nonnenmacher, E. Weibel (eds.), *Fractals in Biology and Medicine*, Birkhäuser, Basel-Boston-Berlin.
- Traverso, S. [2006], Does the Physical State of the Cytoplasm Globally Control Cellular Activity? *Riv. Biol./B. Forum* **99**: 23-29.
- Traverso, S. [2008], A Perspective on Cell Communication: Ion Signaling and Percolation. *Riv. Biol./B. Forum* **101**: 331-340.
- Titushkin, I., M. Cho [2009], Regulation of Cell Cytoskeleton and Membrane Mechanics by Electric Field: Role of Linker Proteins. *Biophys. J.* **96**: 717-728.

Understanding Hematopoietic Stem Cell Mobility Pattern through Mathematics

Sanjiban Sekhar Roy¹, Chi-Hsin Hsu², Zhi-Hong Wen², Chan-Shing Lin² and Chiranjib Chakraborty^{2,3*}

Once, Alexander Maximow, a great visionary in hematopoiesis, wrote:

... [U]nder the influence of stimulation, they [hemocytoblasts, aka stem cells] can be mobilized and become transformed into free, wandering ... elements. ... (Maximow [1924]).

Alexander Maximow was the first scientist who offered the idea of circulating stem cells. Normally, in bone marrow, hematopoietic stem cells can be found. Some percentage of these cells continually escapes of bone marrow and circulates in peripheral blood. The occurrence of hematopoietic stem cells in circulation was experimentally established afterward (Goodman and Hodgson [1962]; Cronkite [1997]). Some molecules with different cellular targets and different biologic activities may help to increase the motion of stem cells. The treatments of the molecules (e.g. endotoxin (Vos *et al.* [1972]; Cline and Golde [1977]), proteases (Vos *et al.* [1972]; Feher and Gidali [1987]), vaccines (Monette *et al.* [1972]), Epo crude preparations (Quesenberry *et al.* [1979]), polymethacrylic acid (Vos *et al.* [1972]), poly I-C (Quesenberry *et al.* [1979]; Mangalik and Robinson [1978]) etc.) can also help to increase the motion, especially the circular motion of bone marrow derived stem cells. In fact, they trigger the mobilization of stem cells from bone marrow. Early studies showing the process of mobilization was quite uncertain. Later, it was certified about the several important conclusions on stem cells and also demonstrated that stem cells can be forced out of bone marrow. The studies established that the mobilizable population from bone marrow is much bigger than the circulating population (Rubin *et al.* [1977]; Fliedner [1998]). Another study concluded that half-time of circulating population is $T^{1/2} = 1-2$ hours (Dorie *et al.* [1979]). The time is very short compared with all other leukocytes movement time. The circulating stem-cell population may be doing balance with bone marrow stem-cell population (Gerhartz and Fliedner [1980]). It was also

recorded that the kinetics of mobilization (i.e., speedy or delayed mobilization (Vos *et al.* [1972])) of stem cells are different and may be varied for one study to another. However, several studies concluded about faster revival after transplantation of mobilized cells (Korbling *et al.* [1995]) or about the fate of mobilized cells held true under a new experimental illumination (Wright *et al.* [2001]; Abkowitz *et al.* [2003]). Although the above studies demonstrated the type of circulation of stem cell and trafficking of circulation of stem cell from bone marrow, it is not clear about the path and pattern of circulation like one dimensional movement or two dimensional movements etc. Mathematics has helped us learn how a cell moves (Brokaw [1990]; Dembo [1989]) and can also count molecules in its environment (Berg and Purcell [1977]). Mathematical models of stem cell movement can significantly alter the way we think about and the type of study. The models can also help us to understand the kinetics of stem cell movement. Here, we discuss the mobility pattern of stem cell by using four mathematical models, which are – one dimensional random walk (1D RW) model; random walk lattices model; two dimensional random walk (2D RW) simulation; two-dimensional circular walk (2DCW) movement, three dimensional random walk (3D RW) simulation and left-right rotational movement.

Random walk movement (1D RW) model can be implemented when a series of stem cells move. The directions are randomly decided for the stem cell movement. Oftentimes the random walk is indexed by the natural numbers such as X_0, X_1, X_2 and also some walks take their steps at random times with the position X_t as $t > 0$. If a stem cell can take one dimensional random walk, it may progress through forward or backward movement (Fig. 1A). Let k be the number of equal steps along a line, say a right step takes h_1 probability and a left step takes h_2 probability, k_1 and k_2 be the number of steps for moving right and left of stem cell, then the relation among them is $h_1 + h_2 = 1$ and $k_1 + k_2 = k$, we can find the probability of taking k_1 steps of stem cell if it moves to the right direction, and we can take $\binom{k}{k_1}$ number of steps for right and left direction. Here $\binom{k}{k_1}$ is nothing but a binomial coefficient, so $P(k_1) =$

Another model is the two dimensional random walk (2D RW) model. Actually, moving a stem cell will usually stop for a period of time before continuing on its walk, or it may divide, followed by walks of both daughter cells. This model can be implemented during such situation. Lee *et al.* [1995] tracked individual EC motion experimentally in 2D – average cell speed, duration of time remaining stationary, and average direction changes were determined for use as parameters in simulations. Lee describes that this distinct system provides another approach to continuous models that use normal and partial differential equations to describe the dynamics of systems evolving in space and time (Lee *et al.* [1995]). This distinct model can be used to describe movements of individual stem cells rather than looking at entire populations of cells. We can calculate the possible 2D orientation of stem cell also using vectors. If, say, k is the number of two dimensional vectors for random orientations, then all vectors movements for k arbitrary directions make an angle θ , where θ varies in $[0, 2\pi]$ range in a complex plain and k step can be shown as

$$Z = \sum_{j=1}^k e^{i\theta_j}$$

Now calculating its absolute square

$$|z|^2 = \sum_{j=1}^k e^{i\theta_j} \sum_{m=1}^k e^{-i\theta_m} = \sum_{j=1}^k \sum_{\substack{m=1 \\ m \neq j}}^k e^{i(\theta_j - \theta_m)} = k + \left\langle \sum_{\substack{j,m=1 \\ m \neq j}}^k e^{i(\theta_j - \theta_m)} \right\rangle$$

So we can write the above expression as

$$\langle |z|^2 \rangle = k + \left\langle \sum_{\substack{j,m=1 \\ m \neq j}}^k e^{i(\theta_j - \theta_m)} \right\rangle$$

If we consider the distribution values of the displacement (θ_j , θ_m) of the stem cells (both positive and negative direction movement can be possible), their average (expected) is zero.

Therefore

$$\langle |z|^2 \rangle = k$$

In that case, rms (root means square) distance will be

$$|z|_{\text{rms}} = \sqrt{k}$$

So, for s step size the distance becomes

$$b_{\text{rms}} = s \sqrt{k}$$

Stem cells can have circular walk, meaning that the movement is on the trajectory of the circle, where the two-dimensional circular walk (2DCW) movement can be implemented. The circular path can be represent as a simple Euclidean geometry (Thomas [1956]; Tsutsumi [1992]) consisting of those points (X_n, Y_n) in a plane which are the same distance $\left(\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}\right)$ from the given point called the center.

Cheng, one of the pioneer researcher from the same research group of Lee, explored the purpose of random walk model of cell motility in three dimension (Cheng *et al.* [2006]) (3D). This model supposes a highly porous scaffold which permits unrestricted motion (Fig. 1C). The algorithm for 3D motion is much related to that of 2D motion. However, it is also containing a migration index, stem cell division counter, direction persistence counter, waiting time, and varying transition probabilities to determine the new direction that a stem cell will move in after stopping, colliding, or dividing. Another feature of the model is that it added in a waiting time that a stem cell will remain stationary after colliding with another stem cell, which accounts for the tendency of stem cells to form clusters in 3D. Cell seeding in two modes is considered: uniform cell seeding throughout the 3D space, seeding with cells seeded along edges of a cylindrical portion of the entire 3D grid. The simulation runs until the cell volume fraction, $\kappa(t)$, increases to the point that all available sites are occupied by cells. In another way stem cells can move in x, y and z direction which are statistically independent and the square of the distance from the origin to the point (x, y, z) can be shown as $r^2 = x^2 + y^2 + z^2$ (Howard [1983]).

Sometime, a stem cell may move in left-right rotational direc-

tion. Movements of a stem cell may be counterclockwise (left turn), clockwise (right turn), collinear (no turn) (Fig. 1D). If we calculate cross product, in that case, $P_1 \times P_2$ can be interpreted as the signed area of the parallelogram formed by the points (0,0), P_1 , P_2 , and $(P_1 + P_2)$

$$P_1 \times P_2 = \det \begin{pmatrix} x_1 & x_2 \\ y_1 & y_2 \end{pmatrix} = x_1 y_2 - x_2 y_1 = -P_1 \times P_2$$

From cross product we can interpret that if $P_1 \times P_2$ is positive, then P_1 is clockwise from P_2 with respect to the origin (0,0), if cross product is negative then P_1 is counterclockwise from P_2 , therefore $\vec{P_0 P_1}$ is clock wise from directed segment $\vec{P_0 P_2}$ with respect to their common end point P_0 . Therefore, if $P_1 - P_0$ denotes the vector $P'_1 = (x'_1, y'_1)$, where, $x'_1 = x_1 - x_0$, $y'_1 = y_1 - y_0$, in the same way we can define $P_2 - P_0$.

Therefore the cross product is

$$(P_1 - P_0) (P_2 - P_0) = \det \begin{pmatrix} (x_1 - x_0) & (x_2 - x_0) \\ (y_1 - y_0) & (y_2 - y_0) \end{pmatrix}$$

positive result means $\vec{P_0 P_1}$ is clockwise from $\vec{P_0 P_2}$, and if the result is negative then $\vec{P_0 P_1}$ is anticlockwise from $\vec{P_0 P_2}$ (Cormen *et al.* [2009]).

We can conclude that the cell movement persists as long as the cell is alive. Cell mobilization, like bone marrow homing, is a complex biologic process which needs the orchestrated participation of several upstream regulators and downstream effectors. Copresentation of immobilized chemokines / adhesion molecules and their downstream partners presents a roadmap to hematopoietic stem cells within bone marrow by guiding their directed migration.

Currently, no model can completely describe the stem cell or tissue growth process or stem cell mobilization properly, because there are still too many unknowns regarding the process itself. Application of these models in stem cell behavior and treatment of cells as individuals can be advantageous because the complex behavior of cells can be broken down into ingredients. In the

words of Jones and Sleeman [2006]: “by modeling crucial steps as discrete processes, it is then possible to develop individual areas independently of the rest of the model”. Cohen [2004] stated that caution must be used in applying models to living systems because “theoretical understanding is required as a check on the great risk of error in software and to bridge the enormous gap between computational results and insight or understanding”. Until more of the basic biology is known, as well as the math to represent that biology, models will serve as fair predictors for simplified cases of stem cell dynamics and growth. Knowledge in these and other aspects of the biology of stem cell mobilization is expected to pay high clinical dividends in the future.

¹ School of Computing Science and Engineering, VIT University; Vellore, India

² Department of Marine Biotechnology and Resources, College of Marine Science and Division of Marine Biotechnology, Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung, Taiwan

³ Medical Biotechnology Division, School of Bio-Sciences and Technology, VIT University, Vellore-632014, TN, India

* Correspondence author E-mail: drchiranjib@yahoo.com

REFERENCES

- Abkowitz, J.L., A. E. Robinson, S. Kale, M.W. Long and J. Chen [2003], Mobilization of Hematopoietic Stem Cells During Homeostasis and after Cytokine Exposure. *Blood* **102**: 1249-1253.
- Berg, H.C. and E.M. Purcell [1977], Physics of Chemoreception. *Biophys. J.* **20**: 193-219.
- Brokaw, C.J. [1990], Descriptive and Mechanistic Models of Flagellar Motility. In W. Alt and G. Hoffmann (eds.), *Biological Motion*. Lect. Notes Biomath. 89, Springer-Verlag, Berlin, pp. 128-144.
- Cheng, G., B.B. Youssef, P. Markenscoff, and K. Zygorakis [2006], Cell Population Dynamics Modulate the Rates of Tissue Growth Processes. *Biophys. J.* **90**: 713-724.
- Cline, M.J. and D.W. Golde [1977], Mobilization of Hematopoietic Stem Cells (CFU-C) into the Peripheral Blood of Man by Endotoxin. *Exp. Hematol.* **5**: 186-190.
- Cohen, J.E. [2004], Mathematics is Biology's Next Microscope, Only Better; Biology is Mathematics' Next Physics, Only Better. *PLoS Biology* **2**: e439.
- Cormen, T.H., C.E. Leiserson, R.L. Rivest, and C. Stein [2009], *Introduction to Algorithms*. (third ed.). MIT Press, USA.
- Cronkite, E.P. [1997], Response to “Mobilization of Peripheral Blood ‘Stem’ Cells:

- Where there is Smoke, is there Fire?" Yes, there have been Many Fires, Leaving Cold Coals. *Exp. Hematol.* **25**: 185-186.
- Dembo, M. [1989], Field Theories of the Cytoplasm. *Comments Theor. Biol.* **1**: 159-177.
- Dorie, M.J., M.A. Maloney, and H.M. Patt [1979], Turnover of Circulating Hematopoietic Stem Cells. *Exp. Hematol.* **7**: 483-489.
- Feher, I. and J. Gidali [1987], Self-Renewal Capacity of Mobilized Murine Haemopoietic Stem Cells. *Haematologia (Budap.)* **20**: 15-23.
- Flidner, T.M. [1998], The Role of Blood Stem Cells in Hematopoietic Cell Renewal. *Stem Cells* **16**: 361-374.
- Gerhartz, H.H. and T.M. Flidner [1980], Velocity Sedimentation and Cell Cycle Characteristics of Granulopoietic Progenitor Cells (CFUc) in Canine Blood and Bone Marrow: Influence of Mobilization and CFUc Depletion. *Exp. Hematol.* **8**: 209-218.
- Goodman, J.W. and G.S. Hodgson [1962], Evidence for Stem Cells in the Peripheral Blood of Mice. *Blood* **19**: 702-714.
- Howard, C.B. [1983], *Walks in Biology*. Princeton U.P., Princeton (NJ), pp. 1-93.
- Jones, P.F. and B.D. Sleeman [2006], Angiogenesis – Understanding the Mathematical Challenge. *Angiogenesis* **9**: 127-138.
- Korbling, M., D. Przepiorka, and Y.O. Huh [1995], Allogeneic Blood Stem Cell Transplantation for Refractory Leukemia and Lymphoma: Potential Advantage of Blood Over Marrow Allografts. *Blood* **85**: 1659-1665.
- Lee, Y., S. Kouvroukoglou, L.V. McIntire, and K. Zygourakis [1995], A Cellular Automaton Model for the Proliferation of Migrating Contact-Inhibited Cells. *Biophys. J.* **69**: 1284-1298.
- Mangalik, A. and W.A. Robinson [1978], Mechanism of Poly I: Poly C Stimulation of Human Bone Marrow Colony Growth in Vitro. *Proc. Soc. Exp. Biol. Med.* **158**: 151-155.
- Maximow, A.A. [1924], Relation of Blood Cells to Connective Tissue and Endothelium. *Physiol. Rev.* **4**: 533-563.
- Monette, F.C., B.S. Morse, D. Howard, E. Niskanen, and F. Stohlman Jr. [1972], Hemopoietic Stem Cell Proliferation and Migration Following Bordetella Pertussis Vaccine. *Cell Tissue Kinet.* **5**: 121-129.
- Quesenberry, P., K. Levin, K. Zuckerman, N. Rencricca, R. Sullivan, and W. Tyler [1979], Stem Cell Migration Induced by Erythropoietin or Haemolytic Anaemia: The Effects of Actinomycin and Endotoxin Contamination of Erythropoietin Preparations. *Br. J. Haematol.* **41**: 253-269.
- Rubin, P., N.A. Elbadawi, R.A.E. Thompson, and R.A. Cooper Jr. [1977], Bone Marrow Regeneration from the Cortex Following Segmental Fractionated Irradiation. *Int. J. Rad. Oncol. Biol. Phys.* **2**: 27-38.
- Thomas, L. [1956], *The Thirteen Books of Euclid's Elements*. (2nd ed.), Dover Publications, New York.
- Tsutsumi, M., K. Sakai, J.S. Kim [1992], A New Measuring Method of Circular Movement of NC Machine Tools: Development of Alternative Method for Standardization. *Transactions of the Japan Society of Mechanical Engineers C* **58**: 271-276.

- Vos, O., W.A. Buurman, and R.E. Ploemacher [1972], Mobilization of Haemopoietic Stem Cells (CFU) into the Peripheral Blood of the Mouse; Effects of Endotoxin and Other Compounds. *Cell Tissue Kinet.* 5: 467-479.
- Weisstein, E.W. [2010], "Random Walk". From MathWorld – A Wolfram Web Resource. <<http://mathworld.wolfram.com/RandomWalk.html>>.
- Wright, D.E., A.J. Wagers, A.P. Gulati, F.L. Johnson, and I.L. Weissman [2001], Physiological Migration of Hematopoietic Stem and Progenitor Cells. *Science* 294: 1933-1936.

A Fungus, Anastomosis, Diploids and Quantum Mechanics

Michael Lieber

The haploid, multinucleated fungus, *Phycomyces*, has been the subject of research for many years. Though a sexual cycle exists, a parasexual cycle was not observed. A diploid strain or stage was never detected. (For a review, see Bergman *et al.* [1969].) Especially studied was the phototropic behavior of the fungus. A detailed investigation of this began in the laboratory of Max Delbruck. In that laboratory in 1975, I conducted some preliminary investigations of the genetics of *Phycomyces*, with the eventual objective of frequently isolating new strains that would prove useful to the research objectives of the laboratory.

Using a methodology that was applied to another haploid, multinucleated fungus, *Aspergillus nidulans*, but not to *Phycomyces*, where another method was routinely applied, which did not involve hyphal fusion, I created conditions that led to the production of forced heterokaryons in *Phycomyces* on minimal growth media. These isolated heterokaryons, unlike previously studied *Phycomyces* heterokaryons on minimal media, had unstable characteristics, including high-growth rate sectoring, which suggested instabilities in gene expression or frequent and variegated genomic changes throughout the mycelia of the heterokaryons. Also found or detected, through strains isolated from the heterokaryons, was evidence of genetic recombination or chromosomal re-assortments (Lieber [1975]).

Such re-arrangements, as made evident through the re-organized genetic markers in the isolated strains via spores, would indicate the formation, through the fusion of haploid nuclei in the heterokaryons, of transient or unstable diploids; which, within the heterokaryons, underwent a haploidization process similar to what occurs through the parasexual cycle via diploids isolated from heterokaryons of *Aspergillus nidulans* (Roper [1952]; Pontecorvo *et al.* [1953]). The eventual result in *Aspergillus* would be the production of haploid, recombinant spores and haploid sectors growing from such. The sectoring noted in *Phycomyces* might have been the phenotypic manifestation of successive chromosome loss from a diploid state leading to variations of nuclei within regions of the

heterokaryotic mycelia. One might have predicted from this that some specific circumstances, as opposed to others, could have again led to the production of unstable diploids in *Phycomyces* heterokaryons or to heterokaryons containing nuclei of various chromosome numbers resulting from the unstable diploids.

In point of fact, much later research demonstrated the generation of diploids and a haploidization process through a parasexual cycle in *Phycomyces*, leading to tissue of multiple and variegated nuclei (Mehta and Cerda-Olmedo [2001]). Specifically, through random chromosomal losses from a *Phycomyces* diploid during its vegetative growth, nuclei of various ploidy and numbers were produced in mycelia. From such mycelia containing multiple and variegated nuclei, heterokaryons developed. These in turn produced mycelia sectors having complex, variegated mixtures of nuclei that had been produced through the haploidization.

Using the methodology that I applied to make the forced heterokaryons in 1975, an anastomosis between the multinucleated hyphae of different haploid strains had to have occurred. However, Professor Delbruck told me many times, "There is no anastomosis in *Phycomyces*." The implication being that anastomosis does not and cannot exist in *Phycomyces*. Using the same general procedure, however, I repeatedly generated heterokaryons of unusual genetic properties and phenotypes. Nevertheless, Professor Delbruck ascribed this to "repeated contamination" from another heterokaryotic source, even though the genetic data did not support his *ad hoc* hypothesis.

Some weeks later, however, Max told me that the surgical technique that I had repeatedly used via a scalpel and forceps may have ruptured the hyphae of the different fungal strains, allowing them to fuse, an anastomosis, thereby resulting in the heterokaryosis, which was repeatedly observed. I considered this to be a very likely explanation, as it appeared to be an inclusive, realistic resolution of two contradictory or inconsistent situations. Years later, after reading extensively about the principles of quantum mechanics, I came to realize the deeper significance or implication of what Max had postulated regarding the non-intended consequences of my surgical technique. In the very process of creating or initiating the experiment, the experimenter, through his methodology or experi-

mental setup, affected or altered the subject within the experiment, and, in so doing, the very observation that could be made. Had I not applied my technique, anastomosis would not have occurred, and thus no observation of such could have been made in *Phycomyces*. By applying my technique, I created a situation leading to anastomosis in *Phycomyces*, which would thus allow the observation or necessary inference of such in *Phycomyces*.

In one, experimental situation or methodological setup, anastomosis was allowed or induced, enabling a subsequent heterokaryotic growth process, hence their observation, while in another experimental situation or methodological setup, different from the first, anastomosis was precluded as well as its specific and necessary developmental consequences, namely heterokaryosis, diploid formation and haploidization, thereby preventing the observation of these. From this perspective, *Phycomyces* has both the incapability and capability of generating anastomosis and its developmental consequence. Which of these becomes manifest at a given time would depend on the particular experimental situation or methodology. No experimental situation could be created that would concurrently allow capable-of-anastomosis and its consequence and not-capable-of-anastomosis and its consequence.

This reflects an important principle of quantum mechanics manifested on the macro-level. This is the Principle of Complementarity, first described by Neils Bohr, Max Delbruck's mentor (Bohr [1933-1958]; [1957]). In early experiments, it was found that when light was observed under one type of experimental arrangement, light displayed wave properties. However, when light was observed under a different experimental arrangement, light displayed corpuscular properties. The two different properties were mutually excluded under any given type of experimental setup, but the two properties complemented to give the complete or actual property of light. Yet no experimental arrangement could be created that showed that light displayed both wave and corpuscular characteristics at the same time, only one to the exclusion of the other of its complementary features. In the quantum realm of physics, there was thus an inability to observe the complete situation with any specific experimental arrangement or methodology, but only a complement of such, and that the particular comple-

ment that could be observed or made manifest depended on the given experimental arrangement or procedure. Neils Bohr referred to this as a Principle of Complementarity operating within the level of quantum mechanics.

I do not know whether or not Max had a complementarity process explicitly in mind when he presented his hypothesis regarding my results. I do know that he and others tried to find an example of the complementarity principle at the biological level by studying the phototropic behavior of *Phycomyces*. Such was not discovered according to what was specifically sought, even though various, generated mutations exist that respectively and apparently alter the phototropic behavior in respective, mutually exclusive ways, depending on which individual, mutant gene is present in the organism (Palit *et al.* [1989]). This in itself may, nevertheless, be some example of a complementarity principle operating through complementary, variable phenotypic expressions of different mutant genes in a photo environment.

The experimenter creates the particular phototropic behavior, excluding all others, by introducing the specific mutant gene into the fungus. Yet, every time one conducts or creates an experiment through a particular experimental setup, manipulation and materials, one is creating a particular situation that excludes all others at that time, but is a complementary part of a more complete reality. Hence, in the very conducting of an experiment, be it on the micro- or macro-level, one is demonstrating the Principle of Complementarity. Experimenting on the biological organism, it would appear, enables the clear demonstration of this principle on the macro-level.

By conducting many different, complementary experiments and obtaining complementary observations, a more complete knowledge of reality should ensue. In this regard, what must a scientist determine? A scientist should determine the features which the various complementary, observed situations or results have in common. These would be the constants transcending the boundaries of the complementing situations. These constant, transcending features would be the objectivity one seeks in scientific research. Such features would become more and more evident or manifested as more complete knowledge arises through the joining of ever more,

complementing experimental situations or approaches. With regard to *Phycomyces*, diploid formation and haploidization are the processes constant across two complementary experimental situations created 25 years apart. The potential for such may become even more evident or manifest through the creation of other, complementary experimental situations, while not evident in others, hence the view held for many years that diploids also do not exist in *Phycomyces*. Thus, an experiment through which diploids were not detected shaped only an incomplete picture.

Each biological experiment performed would appear to allow to be shaped just one part or facet of a more complete reality of biological processes. In this regard, might it be suggested that Max Delbrück and I, and all biological scientists, through their very biological experimentation, made manifest a principle of complementarity beyond the quantum realm. In view of this, one might ask does the biological process itself demand some principle of complementarity for its very existence. Is a completion process or dynamic essential for the life process and does this completion dynamic operate on and through all scales of reality, including the psychological, as a type of dynamic fractal? Perhaps, if science attempts to answer these questions, first by clarifying the completion dynamic in different contexts, a more complete insight into existence would be achieved. From this perspective, the complementation process influenced or shaped by experimental circumstances, whether on the quantum level or on the macro-level, could be a limiting case of that completion dynamic operating in nature. When an experiment is conducted, the completion dynamic could become arrested or stabilized at a specific, incompleting stage, thereby giving rise to incomplete information. If the completion dynamic can be investigated inclusively and successfully from all perspectives, Max Delbrück's objective and that of his mentor, Neils Bohr, to demonstrate the operation of complementation in life will have been achieved on a profound level.

E-mail: michaellieber@juno.com

REFERENCES

- Bergman, K. *et al.* [1969], *Phycomyces*. *Bacteriol. Rev.* **33**: 99-157.
- Bohr, N. [1933-1958], *Foundations of Quantum Physics II. Collected Works*. Elsevier, Amsterdam.
- Bohr, N. [1957], *Atomic Physics and Human Knowledge*. Wiley and Sons, New York.
- Lieber, M.M. [1975], Observations on Forced Heterokaryons in *Phycomyces*. *Caltech Ann. Rev. of Biol.* **23**: 25-26.
- Mehta, B.J. and E. Cerda-Olmedo [2001], Intersexual Diploids in *Phycomyces*. *Genetics* **158**: 635-641.
- Palit A. *et al.* [1989], System Analysis of *Phycomyces* Light-growth Responses: madC, madG, and madH Mutants. *Biophys. J.* **55**: 519-526.
- Pontecorvo, G. *et al.* [1953], The Genetics of *Aspergillus nidulans*. *Adv. Genet.* **5**: 141-238.
- Roper, J.A. [1952], Production of Heterozygous Diploids in Filamentous Fungi. *Experientia* **8**: 14-15.

RISVOLTI / FLAPS

Henry F. Osborn, *Dai greci a Darwin. Disegno storico dello sviluppo dell'idea dell'evoluzione*. Robin, Roma 2010, pp. 222, € 15,00.

Questo saggio di Osborn ebbe origine da una serie di conferenze che lo stesso professore tenne alla Columbia University nel 1893, il cui scopo era quello di mettere in luce le particolarità dell'Evoluzionismo pre-darwiniano, spesso trascurate o mal interpretate.

Osborn ci accompagna così nel lungo viaggio delle teorie scientifiche dell'Evoluzione, illuminando le sue origini lontane e fornendoci una visione olistica e comparata secondo cui "l'Evoluzione ha raggiunto la sua attuale pienezza mediante lente aggiunte in ventiquattro secoli. Quando si riuniscono le verità e le assurdità della speculazione e dell'osservazione greca, medievale, e di quella dal secolo XVI al XVII, appare chiaro che formano un tutto continuo, che l'influenza del pensiero primitivo sul pensiero moderno è più grande di quanto si credesse, che Darwin deve ai Greci più di quanto noi abbiamo mai riconosciuto".

Alla domanda "da dove nacque la vita?" hanno provato a rispondere tutti in ogni tempo e in ogni dove. Noi in queste pagine possiamo scoprire le prime intuizioni, i primi abbozzi delle teorie dei grandi pensatori, filosofi, letterati, naturalisti, astronomi, come Aristotele, Lucrezio, Agostino e Giordano Bruno, Lamarck, Descartes, Diderot e Kant.

Giovanni F. Azzone, *Perché si nasce simili e si diventa diversi? La duplice nascita: genetica e culturale*. Bruno Mondadori, Torino 2010, pp. 205, € 16,00.

Nel mondo biologico, il termine evoluzione è stato quasi sempre associato alle teorie scientifiche di Darwin fondate sull'ereditarietà, considerate un modello valido ed esclusivo anche per gli esseri umani. Contestando questa radicata convinzione determinista, il volume dimostra con un linguaggio chiaro – attraverso temi quali l'intenzionalità, la coscienza, la distinzione tra nascita genetica e culturale – che un processo di evoluzione darwiniano fondato esclusivamente sui geni non è sufficiente a spiegare le trasformazioni del mondo umano.

Il sistema mente-cervello della nostra specie è infatti all'origine di qualcosa di completamente nuovo e di straordinaria potenza: il sistema delle reti neurali, dotato di una fantastica e imprevedibile capacità di generare e comunicare l'informazione, e destinato a creare strutture e attività che non hanno niente in comune con quelle fondate sulle proprietà del DNA e sui meccanismi prevedibili di origine genetica.

Joseph Seckbach and Martin Grube (eds.), *Symbioses and Stress. Joint Ventures in Biology*. Springer, Dordrecht, Heidelberg, London, New York 2010, pp. 630, € 155.95.

Symbioses and Stress centers on the question of how organisms in tight symbiotic associations cope with various types of abiotic and biotic stress. In its original sense, symbioses cover all kinds of interactions among unrelated organisms, whereas in a narrower concept, the term is often referred to as mutualism. Evolutionary biology recognizes symbiosis as an integrative process, and most fundamental evolutionary innovations arose from cooperative symbioses. Mutualisms contribute to stress tolerance, ecosystem stability, and evolutionary radiation of cooperating organisms. Modern eukaryotic cells are the result of the endosymbiotic union of prokaryotic ancestors as well as diverse exosymbiotic associations. This cooperative aggregation appears more successful than its independent parts. This new book presents functional and evolutionary aspects of mutually beneficial symbioses among unrelated organisms.

Nico Stollenwerk and Vincent Jansen, *Population Biology and Criticality. From Critical Birth-Death Processes to Self-Organized Criticality in Mutation Pathogen Systems*. World Scientific, Singapore 2010, pp. 236, USD 96.00.

The present book describes novel theories of mutation pathogen systems showing critical fluctuations, as a paradigmatic example of an application of the mathematics of critical phenomena to the life sciences. It will enable the reader to understand the implications and future impact of these findings, yet at same time allow him to actively follow the mathematical tools and scientific origins of critical phenomena. This book also seeks to pave the way to further fruitful applications of the mathematics of critical phenomena in other fields of the life sciences.

Contents: From Deterministic to Stochastic Dynamics; Spatial Stochastic Birth-Death Process or SIS-Epidemics; Criticality in Equilibrium Systems; Partial Immunization Models; Renormalization and Series Expansion: Techniques to Study Criticality; Criticality in Measles Under Vaccination; Evolution to Criticality in Meningococcal Disease.

Erling Norrby, *Nobel Prizes and Life Sciences*. World Scientific, Singapore 2010, pp. 336, USD 68.00.

The Nobel Prizes in natural sciences have developed to become a unique

measure of scientific excellence. Using archival documents, which have been released (50 years secrecy) for scholarly work, the author expertly traces the strengths and weaknesses of the Nobel system as exemplified by individual prizes. Surveys of the more than 100 years that the Prizes have been awarded are also presented.

This book discusses the most important prize in the world of science and gives unique historical insights into how the laureate selection process has developed to secure optimal choice.

No other book has been published which draws from previously classified archival materials to the extent that this book does. It indirectly deals with factors that foster scientific discoveries viz. the role of both individuals and institutions and thus provides invaluable insights for researchers, institutions and anyone interested in science.

Contents: More than a Century of Nobel Prizes; Serendipity and Nobel Prizes; Nobel Prizes and the Emerging Virus Concept; The Only Nobel Prize for a Virus Vaccine: Yellow Fever and Max Theiler; Polio and Nobel Prizes; Unusual Nobel Prizes in Physiology or Medicine; Nobel Prizes and Nucleic Acids: A Drama in Five Acts; Nobel Prizes, Prions and Personalities.