Tensegrity, Dynamic Networks and Complex Systems Biology:

Emergence in Structural and Information Networks within Living Cells

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1. INTRODUCTION: MOLECULAR CELL BIOLOGY AND THE SCIENCE OF "COMPLEX SYSTEMS"

A major goal of the study of complex systems is to formally describe and understand how a large number of different *parts* interact and self-organize into a *whole* system that exhibits properties that cannot be understood by studying the components in isolation (Bar-Yam, 1997). This goal transcends various levels of description and is of particular importance in biomedical research because living organisms extend over multiple levels of organization. They are hierarchical structures that integrate their smallest constituent parts –individual molecules including DNA, proteins and lipids – across multiple levels of organization, from organelles, to cells, tissues, organs, and the organism (Fig 1). Thus, in biology, it is necessary to traverse multiple levels of organization, from the micro-level of the parts through many iterations of integration in order to understand the whole system at the "macro"-level.

The advent of recombinant DNA technology, almost four decades ago, and the concomitant progress in protein biochemistry have led to great advances in our understanding of the lowest level of organization, the genes and molecular parts that comprise living systems. Analysis of how these components interact has led to the elucidation of fundamental principles of living cells, such as the genetic code, the transcription of genes into mRNA and translation of mRNA into proteins. Since then the unfathomable complexity of other molecular processes of livings systems, such as the cell's growth cycle and regulation of its behavior by external signals, has attracted most attention in biology. Now most molecular biologists almost entirely focus their efforts on

the identification of new genes and proteins, and characterization of the role of these components in these control processes.

Recently, it has become clear to some in the field that rather than studying individual proteins separately, an integrative approach is necessary. This is reflected in the burgeoning area of "Systems Biology" (see Chapter [Hood Chapter]) which seeks not only to systematically characterize and categorize all the molecular parts of living organisms using massively-parallel analytic techniques, but also to understand the functional interactions between the molecules using computational approaches. However, despite these efforts, most researchers still use the new high-throughput technologies of genomics to accelerate and expand to the genome-scale the discovery of new molecular pathways. In contrast, the importance of vertical integration across different levels of organization is still largely neglected.

Moreover, in molecular biology, it is still often assumed that the ability to describe mechanistic details through experimentation or use of mathematical models is equivalent to "understanding" the behavior a complex system. For instance, a cell is thought to divide because a growth factor binds to a cell surface receptor, activates a biochemical cascade (e.g., ras-raf-MEK, Erk), triggers expression of the protein cyclin D1; this phosphorylates and thereby inactivates the cell-cycle inhibitor protein Rb which, in turn, leads to the induction of proteins involved in DNA replication (Sherr, 1994). Although we present this type of Rube Goldberg-like mechanism of a molecular process in a living cell as if it were explanatory, the reality is that such mechanistic representations are essentially descriptive. All we do is describe a chain of events at a lower (molecular) level than the one used to make our initial observation (cell or tissue

level). Even formal and quantitative approaches like systems dynamic modeling of particular molecular pathways are descriptive because they use an established set of rules (e.g. formal reaction kinetics and diffusion) to describe and predict in detail the time evolution of a *particular instance* of a system whose generic behavior is known given the set of equations and conditions. None of these approaches meet the challenge of integration by transcending the various levels organization.

In view of the rapid rise of molecular biology and genomics, some biologists did voice caution about the limitations of this descriptive and reductionist stance (Ingber and Jamieson, 1985; Schultz, 1996; Strohman 1997; Ingber 1998;, Rose 1998; Coffey 1998; Lewontin 2000), calling attention to Aristotle's insight that the "whole is different from the sum of its parts". However, it is only now, at the threshold of post-genomic and systems biology, that life scientists are beginning to realize that an accurate description of all the parts that comprise a living cell is not equal to understanding how it functions (Huang, 2000). Sometimes capturing the impression of the whole picture with a glance can give deeper insights and yield information not obtained by reproducing it pixel by pixel. Although biologists have yet to adopt the approach of "coarse-graining" to gain insight into fundamental, system-wide properties, this method is often used by physicists (Gell-Mann 1994; Weiss et al., 2003). Only by adjusting our analysis to focus on various levels of organization, and "zooming" in and out on the magnification, can we reveal the fundamental principles that govern what makes the whole (the organism) different from the sum of its parts (the molecules).

To do so, biologists must free themselves from their "divide and conquer" mentality and their adherence to molecular description as the only mode of explanation.

Instead, they must join physicists in their willingness to embrace abstraction and generalization. Conceptual and formal tools are also needed that go beyond descriptive mathematical modeling of particular molecular pathways. At the same time that the molecular biologist now faces this new challenge, the science of "Complex Systems" appears to have matured as a discipline in its own right. Even if a rigorous scientific underpinning remains to be established, it has encouraged scientists from various fields, such as physics, biology, engineering, business and the social sciences to join forces and take a formal approach to complex systems in their subject of interest rather than creating models that only reenact all of its details.

A specific formalism that is particularly useful here is based on the idea that a complex system can be treated as a *network* of interacting parts in a most general sense. A network can be a physical or mechanical structure, as well as an abstract representation of how information flows between interacting elements within a system. Thus, in this chapter, we focus on the principles of organization that govern how cells control both their physical structure and biochemical function as a result of interactions within underlying networks of interacting of proteins and genes. In the process, we will describe how simple, rule-governed behaviors, such as cell shape stability and cell fates, represent "emergent properties" of the underlying molecular networks. Finally, we raise the possibility that the interface between these two networks, one structural and the other informational, is at the core of evolution and functioning of complex living systems, such as cells and whole organisms, that operate at size scales much higher than that of molecular reactions and flows.

2. COMPLEXITY IN LIVING SYSTEMS

How does the information encoded within DNA and biochemical reactions map into the observable properties of living cells that comprise all organisms? This old riddle of the genome-phenome relationship can be split into two more specific questions: 1) How do interactions between biochemical components lead to the production of a physical object with distinct structural properties characteristic of a living cell?, and 2) How do interactions among genes, proteins and cells lead to the development of a coherently functioning machinery for information processing that governs how living cells will behave and adapt to their surroundings? (Fig. 1) Thus, we need to address the question of how higher level behaviors emerge, in the context of both the hardware (structure) and the software (information processing programs) of the cell.

In contrast to simpler, widely discussed emergent phenomena, such as pattern formation in physico-chemical systems or patterns of animal flocks, the emergence of new properties in cell biology has two particular characteristics which are absent in most non-living complex systems:

•*Hierarchy of multiple levels of emergence.* New properties emerge at multiple hierarchical levels that cover many size scales in living organisms. Genes and amino acid sequences determine protein structure, i.e. their three-dimensional (3D) shape and mechanical properties. Proteins and other macromolecules (e.g., lipids, nucleic acids) self-assemble to create functional multi-molecular complexes and intracellular organelles, such as the mitochondrion, nucleus, and plasma membrane as well as the extracellular matrix (ECM). These organelles join together with other biofunctional molecular scaffolds and the surface membrane to form cells. Cells interlink with each

other and with extracellular matrix to form tissues. Multiple tissues combine to form organs that, in turn, are linked together to form the organism.

As the elements at each level (molecules, organelles, cells, tissues, organs) interact, they give rise to emergent properties that are characteristic of the next higher level. For example, individual proteins may exhibit a low level of catalytic activity and move free in solution. However, when multiple proteins with different enzymatic activities assemble together they can form a higher order enzyme complex which exhibits stable 3D form as well as novel functions based on coupled metabolic processing activities. For example, the pyruvate dehydrogenase enzyme complex has a mass approaching 10 million Daltons in mammals and it exhibits a highly organized, pentagonal dodecahedral shape (Wagenknecht et al. 1991) . Similarly, individual cells of the pancreas can secrete digestive enzymes in a polarized manner (i.e., from the apical pole of the cell), however, disease (pancreatitis) results if these cells dissociate from each other and their orienting extracellular matrix scaffold, and lose their higher order tissue architecture. Thus, each level has specific rules of interaction that involve structural as well as dynamic constraints since the nature of the parts and interactions of each level are different. Therefore, unlike fractals, we have discrete layers of patterns governed by distinct rules, and there is no general "scale-invariance" although some principles apply to various scales, as we will see.

•*Heterogeneity of interacting elements.* The emergent entities (e.g., individual molecules with characteristic 3D structure and function, multimolecular complexes with novel enzyme activities, organelles with specialized metabolic functions, living cells that move and grow) do not form a uniform population, as is the case for the molecules in

self-organizing patterns of chemicals or of individuals in schools of fish. Instead, these entities are unique individuals, or belong to classes of entities with similar properties that can be clearly distinguished from each other. For instance, cells that arise from molecular self-assembly can be classified into hundreds of qualitatively different classes or types (e.g., liver, muscle, nerve, skin, etc.), even though they are composed largely of the same molecular components.

The complexity of each biological network enables combinatorial diversity; hence, this leads to individuality, even though each network is comprised of similar interacting parts. Individuality of the parts in turn enables combinatorial diversity at the next level. This increases the variety of interaction rules at each size scale, thus adding a unique layer of complexity characteristic of living organisms. As mentioned earlier, it is the heterogeneity and size of the population of the molecular parts that necessitates both the massively-parallel, descriptive methods and the detailed modeling approaches of systems biology. However, systems biology does not currently include a mechanism to handle or explain hierarchical complexity.

3. NETWORKS AS A GENERAL CONCEPTUAL FRAMEWORK

Given that the cells are the most basic building unit of life, we will first focus our discussion on how cell shape and function emerge from interactions among thousands of interacting molecules and genes. Our goal is to uncover principles that govern how many, heterogeneous, interacting molecular components can self-assemble to produce higher level features characteristic of whole living cells. However, as we will show, the same design principle may also govern how emergence occurs at higher levels of

organization (e.g., tissues, organs, organism), even though the higher level networks are composed of different players with distinct rules of interaction.

In chemistry, aggregate variables may be used to represent an average property of a homogenous population of parts. Unfortunately, because of the various levels of emergence and the heterogeneity of the parts in a whole organism, this approach is not well suited to describe the complexity of living systems. In fact, this is the major limitation in most past studies that attempt to explain cell structure and mechanics using conventional engineering approaches (e.g., continuum mechanics), as well as cell function using laws of mass action for molecular interactions.

In contrast, networks provide a simple general formalism for understanding how the collective action of interacting parts gives rise to emergent properties, and thus a means to handle hierarchical complexity. Because the essential ingredients that make the whole different from the sum of its parts are the *interactions* between the heterogeneous components, a biological system can be formalized as a large *network* that consists of the component elements (the molecules) and their links (their interactions) which need not be identical. The major point here is that network models can be applied to both *structural* systems (i.e., physical scaffolds that lend mechanical stability to the network) and *information processing* systems (i.e., the abstract diagrams that represent how elements of the network influence each other's activities and the behavior of the whole). Applied to mammalian cells, the structural network is the "cytoskeleton" that determines how the building blocks (proteins) are physically attached to each other to give the cell its physical shape and mechanical stability. The information processing network is the regulatory network that determines how the state of interacting elements (genes and

proteins) influence each other, and thereby process the information that is encoded in the genome or received from the external milieu to generate a distinct cell behavior.

The common basic property of both networks in living cells, one structural and the other informational, is that they “self-organize” from their constituent molecular elements to form a stable system with emergent properties. However, there are some formal differences. The structural network model describes a concrete object while information networks are an abstraction. Nevertheless, while one may think of the structural network as a physical scaffold in which every individual building block (including all members of the same class of elements) has to be depicted in the model, like in an architectect’s blueprint, the architectural model of the cell that we discuss below also offers some abstraction. Specifically, models that contain a few elements (with their prototypic mechanical properties) mimic characteristic global properties of whole living cells that contain millions of such elements. On the other hand, information processing networks are full abstractions in that every individual interacting element that occurs only once in the model actually represents hundreds to billions of copies of that particular type of molecular species. Another difference between the structural and information networks is that the former takes into account position and physicality, whereas interactions in the latter can be represented mathematically as a graph because, in a first approximation, space does not play a role.

At first glance, one might think that the structural cytoskeletal network maintains the cell’s shape, whereas the information networks (e.g., signal transduction networks) determine the behavioral state of the cell. However, as will be discussed below, evolution has led to assembly of cells in which structure and information processing

functions are tightly coupled; this turns out to be a fundamental property of living systems at all size scales. Another central property of biological systems, such as the cell, is that they need to be stable, yet flexible. Cells are continuously challenged by chemical and physical stimuli: not only do cells have to resist random perturbations and maintain their structure and behavioral program they also have to be flexible enough to respond appropriately to specific external signals that require distinct changes in both cell mechanical and biochemical behaviors. Interestingly, death of both cells and whole organisms is characterized by a rapid increase in rigidity (*rigor mortis)*, with a complete loss of the flexibility that dominates the living state. Thus, this unification of *robustness* with *flexibility,* both in terms of cell structure and behavior, is a hallmark of complex living systems.

4. STRUCTURAL NETWORKS IN CELLS

4.1. From molecular biochemistry to cellular mechanochemistry: the cytoskeleton

Cells are comprised of thousands of molecules that are arranged and connected in specific ways so as to produce distinct structures and biochemical functions; they are not membranes filled with viscous colloidal solution. In particular, mammalian cells contain an internal molecular framework or “cytoskeleton” that provides shape stability to the cell, and orients much of the cell’s metabolic and signal transducing machinery (Ingber, 1993a; Ingber, 1993b). The cytoskeleton is an interconnected 3D network or lattice comprised of three major classes of filamentous protein polymers – microfilaments, microtubules and intermediate filaments. A subset of the microfilaments

that contain myosin as well as actin are contractile; these filaments actively generate mechanical tension through a filament-filament sliding mechanism similar to that used in muscle (Ingber, 1993a; Ingber, 2003a). Thus, the entire cytoskeleton and cell exists in a state of isometric tension. In essence, by organizing this multimolecular network, the cell translates a structureless chemistry into a physical entity with well-defined mechanical properties. For example, this tensed intracellular scaffold is largely responsible for the viscoelastic properties of the cell. It also generates the tractional forces that drive cell movement as well as changes in cell shape.

4.2. Cellular Tensegrity

Past work on cell shape and mechanics ignored the cytoskeleton and assumed that the cell is essentially an elastic membrane surrounding a viscous or viscoelastic cytosol (Evans and Yeung, 1989; Dong et al., 1991; Fung and Liu,1993).In contrast, over the past twenty years, we and others have been able to show that the cytoskeleton is the major determinant of cell shape and mechanics, and that cells may use a particular form of architecture known as “tensegrity” to organize and stabilize this molecular network (rev. in Ingber, 2003a).

Tensegrity was defined by Buckminster Fuller as a building principle in which structural shape of a network of structural members is guaranteed by continuous tensional behaviors of the system and not by local compressional member behaviors (Fuller, 1961). The purest representation of the tensegrity principle is found in the creations of the sculptor, Kenneth Snelson, which are composed of a continuous network of high tension cables and a discontinuous (isolated) set of compression struts (Fig. 2). However, the tensegrity principle also applies to all geodesic structures

(Ingber, 1998; Ingber, 2003a). Tensegrity networks have the property that they are self-stabilizing in the sense that they yield equilibrium configurations with all cables in tension only due to the internal interactions between its components, i.e., without the need for external forces. Specifically, the tension members pull against the resisting compression members and thereby create an internal tensile stress or "prestress" (isometric tension) that stabilizes the entire system (the same prestress may be generated by the compression members pushing out against a surrounding resistance network). Moreover, both multimodular and hierarchical tensegrity networks can be created that are governed by the same rules and that exhibit integrated system-wide behaviors when exposed to external stress (Ingber, 2003a).

In the cellular tensegrity model, the whole cell is a prestressed tensegrity structure, however, geodesic structures are also found in the cell at smaller size scales (Ingber, 1998; Ingber, 2003a). In the model, tensional forces are borne by cytoskeletal microfilaments and intermediate filaments, and these forces are balanced by interconnected structural elements that resist compression. These latter elements include microtubule struts within the cytoskeleton and cell surface adhesions to the surrounding extracellular matrix. However, biological systems are dynamic and highly complex in that individual filaments can have dual functions and hence bear either tension or compression in different structural contexts or at different size scales. The tensional prestress that stabilizes the whole cell is generated actively by the actomyosin apparatus within contractile microfilaments. Additional passive contributions to this prestress come from cell distension through adhesions to the ECM and other cells, osmotic forces acting on the cell membrane, and forces exerted by filament

polymerization. Intermediate filaments that interconnect at many points along microtubules, microfilaments and the nuclear surface provide mechanical stiffness to the cell based on their material properties and on their ability to act as suspensory cables that interconnect and tensionally stiffen the entire cytoskeleton and nuclear lattice. In addition, the internal cytoskeleton interconnects at the cell periphery with a highly elastic, cortical cytoskeletal network directly beneath the plasma membrane. The entire integrated cytoskeleton is then permeated by a viscous cytosol and enclosed by a differentially permeable surface membrane.

Unlike the isotropic viscous cytoplasm that dominated past models of cell mechanics, the tensegrity-stabilized cytoskeletal network optimizes structural efficiency (strength/mass ratio) by relying on internal tension, rather than on continuous compression when exposed to an external force. Tensegrity systems also can easily change shape with minimal energy consumption, for example, as compared to classical truss structures which require an excessive amount of energy even for minor shape modification. Most importantly, as with all complex networks composed of multiple interacting components, the macroscopic properties of tensegrity networks (e.g., their mechanical stability, ability to grow and rearrange, structural efficiency, viscoelastic behavior) are emergent properties that arise from the particular architecture used to stabilize the 3D network.

4.3. Computational tensegrity models predict complex cell behaviors

If the complex mechanical behaviors of cells, including their global mechanical stability, flexibility, ability to remodel, and optimal strength/mass ratio, represent emergent properties of cell structural networks, then we should be able to get insight into how this

takes place by studying and modeling cytoskeletal mechanics. Existing paradigms assume that the static and dynamic mechanical behaviors of living cells are assumed to respectively originate from two distinct compartments - the elastic cortical membrane and the viscous cytoplasm. Recent work, however, has revealed that cell dynamic behavior reflects a generic system property of the cell at some higher level of molecular interaction as it is characterized by a wide spectrum of time constants (Fabry, *et al*. 2001). Dimitrije Stamenovic working with our group (Stamenovic et al., 1996), and others (Wendling et al., 1999, 2002; Volokh et al., 2000, 2002), have shown that a theoretical formulation of the cellular tensegrity model based on first mechanistic principles can predict various static mechanical properties of living mammalian cells. More recently, we found that the tensegrity model also can explain dynamic cell mechanical behaviors, as described below.

The theoretical tensegrity model of the cell is a deterministic physics-based model which assumes that contractile microfilaments and intermediate filaments carry a stabilizing tensile prestress in the cytoskeleton that is balanced by internal microtubule struts and extracellular adhesions. The cytoskeleton and substrate together were assumed to form a self-equilibrated, stable mechanical system; the prestress carried by the cables is balanced by the compression of the struts. The simplified tensegrity network used in the computational model is composed of 24 tensed, linearly viscoelastic (Kelvin-Voigt), “microfilament” cables and 6 rigid “microtubule” struts; 12 additional tensed Kelvin-Voigt “intermediate filament” cables extend from the surface of the structure to the cell center and the basal ends of 3 struts are fixed to mimic cell substrate adhesion (Fig. 2). Importantly, work on variously shaped models has revealed that even the simplest prestressed tensegrity network embodies the key mechanical properties of all prestressed tensegrities (this is the degree of abstraction mentioned above). In this computational model, the material properties of the tensile filaments can be varied independently. The equilibrium solution around which the linear mathematical

model was derived for frequency response calculations is a prestressable configuration (Sultan, et al., 2001). The prestress is a measure of the tension in the cables. The input was a vertical, sinusoidally varying force applied at the center of a strut; the output was its corresponding vertical displacement (Fig. 2).

Analysis of the variations of the dynamic elastic modulus *G*' and dynamic frictional modulus *G"* with the level of prestress for various frequencies in this computational tensegrity model revealed that these dependencies increased approximately linearly over a wide range of prestress. These results nicely mimic experimental observations that demonstrated the same behavior in living cells (Stamenovic et al., 2002) (Fig. 3) and confirm similar results obtained with a slightly different tensegrity structure (Cañadas, et al., 2002). Deviations from the experimental results were only observed at very low prestress where the cables are almost slack. Adherent living cells actively generate tension within their contractile microfilaments and thus, their cytoskeleton is always prestressed. Importantly, analysis of the frequency dependencies of *G*' and *G*" of the tensegrity structure also revealed a wide distribution of time constants that closely mimicked behavior previously observed in living cells (Fabry et al., 2001) (Fig. 3). Similar results were obtained for other types of loading and for tensegrity structures of higher complexity. However, better results were obtained with heterogeneous models in which different filaments exhibited different levels of stiffness.

Thus, a key feature of the cellular tensegrity network — the level of cytoskeletal prestress — is critical for control of both static and dynamic mechanical behavior in whole cells. As predicted by the model, the global system architecture and inhomogeneity of time constants between individual elements also significantly contributes to the emergent properties of the system: the whole network behaves differently than an individual Kelvin-Voigt cable. This finding that both elastic and frictional behaviors of living cells naturally fall out from the tensegrity model indicates

that the viscous properties of mammalian cells are not due to fluid behavior of the cytosol. Rather, these complex mechanical properties of cells emerge from collective mechanical interactions among the distinct molecular filaments that comprise the cytoskeletal network. These results emphasize the importance of the tensionally-prestressed cytoskeleton for cell mechanical behavior and add further support for the universality of the cellular tensegrity model (Ingber 1998; Ingber, 2003a).

4.4. Biological implications of tensegrity beyond the cytoskeleton

In a more encompassing biological interpretation, a mechanical design principle that uses networks composed of discrete elements rather than a single mechanical continuum allows molecules (e.g., the proteins that form the filaments) to bridge the gap between microscopic structure-less biochemistry and macroscopic mechanics and pattern in just one step of self-assembly. However, as mentioned in the introduction, living systems harbor a hierarchy of many levels of emergence over many size scales. Of interest thus is that the principle of tensegrity is scalable, and in fact operates at various size scales, from molecule to organism (Ingber, 1998; Chen and Ingber, 1999; Ingber 2003a). For example, tensegrity may govern how individual molecules, such as proteins, and multimolecular structures (e.g., lipid micelles) gain their mechanical stability and 3D form (Ingber, 1998; Farrell et al., 2001.; Ingber, 2003; Zanotti et al., 2003). Geodesic forms also are dominant in molecular systems including viruses, the simplest example of a living system; interesting, tensegrity was used to explain the geodesic structure of viral capsids (Caspar, 1980).

At a larger size scale in living tissues, cells are attached to anchoring scaffolds that are also 3D structural networks composed of fibrillar extracellular matrix molecules. Because cells apply cytoskeletally-generated tractional forces on their adhesions, these extracellular matrix networks are also prestressed and hence stabilized through tensegrity. Local increases in tissue tension are sensed by individual adjacent cells which respond by switching into a proliferative state, thereby increasing cell mass to

match increases in applied macroscopic forces. In this manner, tension-dependent changes in cell growth allow higher order tissue and organ structures, such as glandular buds and brain gyri, to be sculpted by organ-level mechanical forces during morphogenesis. Finally, provision of stability and flexibility within a biological network through a tensegrity force-balance is most obvious at the highest level of organization in the hierarchy of life. The musculoskeletal system that allows human to walk and hold our bodies in various positions gains its stability through a balance between continuous tension (muscles, tendons, ligaments) and local compression (bones) that generates a tensile prestress (tone).

Thus, tensegrity appears to represent a fundamental design principle that is used to stablize biological networks at all size scales in the hierarchy of life, as well as throughout evolution (Ingber, 1998). The flexibility and stability provided by use of tensegrity also may have contributed significantly to the process of hierarchical self-assembly and environmental selection that first led to origin of cellular life (Ingber, 2000), as well as to the development of multicellular organisms comprised of interconnected networks of cells, tissues and organs (Ingber, 2003a).

Important, the complex mechanical behaviors of a tensegrity system represent emergent properties of the whole network, and not properties of the individual structural members. For these reasons, tensegrity may provide a means to incorporate "physicality" and spatial constraints into models of complex network systems that commonly are only thought of in terms of information flow. Interestingly, most biochemical reactions proceed in a "solid-state" in living cells, i.e., many of the enzymes, substrates and reactants are physically immobilized on insoluble cytoskeletal scaffolds; (Ingber, 1993b; Ovadi and Srere, 2000; Carpenter 2000). Thus, mechanical properties of structural networks, and hence tensegrity principles, may also directly impact information flow in biological systems, as will be discussed below.

5. INFORMATION NETWORKS

On the hardware side, the mechanical properties of the cells are the obvious properties that emerge from interactions between structural proteins. In contrast, on the software side, the emergence of some simple, fundamental, higher-level features from interactions among regulatory genes and proteins is not immediately apparent. Here we show that despite the complexity of molecular pathways within a cell, global cell behaviors associated with a change of phenotype exhibit simple rule-governed properties that emerge from interactions in the regulatory network of the cell.

5.1. Cell fates as emergent properties

The global behavior of a cell within a tissue in a multicellular organism can be reduced to a few behavioral modes or phenotypes, the so called “cell fates”: proliferation, differentiation, or cell death (apoptosis) (Huang, 2002). During proliferation, cells are in a biochemical state in which they can replicate DNA and divide to increase tissue cell mass. During differentiation, cells undergo a phenotypic change from an immature precursor cell to a distinct cell type, such as red blood cell, liver cell or nerve cell, that carries out tissue-specific tasks. In apoptosis, cells respond to particular signals by switching on a suicide program and undergo cell death. Each cell fate is characterized by a distinct profile of activation of the 30,000 or so genes in the human genome.

Cell fates are stable, mutually-exclusive cellular states (Goss, 1967; Huang, 2002). The conditional selection of these cell fates within the population of cells in a tissue gives rise to the next level of emergence: the tissue and organs which consist of distinct spatial patterns of cells that exhibit different fates, including various specialized cell types. The tissue is a cellular society that requires social behavior of its members in

order to maintain its global structural and functional stability. Thus, the balance between division, differentiation and death of individual cells needs to be tightly regulated within different tissue microenvironments so that the whole tissue optimally responds to all environmental signals.

Cell fate switching is governed by a molecular network of genes, proteins and other cellular components that give rise to the emergent property we recognize as cell fate. For simplicity, let us here focus on the gene regulatory network, and ask the more general question: How can the mutual regulation of >30,000 genes in the genome give rise to stable, mutually-exclusive cell fates, each characterized by a distinct gene activation profile? For example, why does a differentiated liver cell not drift away to become a nerve cell if the difference is just in the pattern of gene activation? As described above, another important property for development is that cells unite stability (maintenance of identity in response to perturbations) with flexibility (ability to change identity in response to critical stimuli); in fact, it is this property that allows development to take place in the first place. This and other qualities are fundamental, emergent properties that, as we will see, arise as a consequence of how information is processed by the underlying gene regulatory network and the architecture of that network.

5.2. Network dynamics leads to stable states: **attractors in gene regulatory networks**

Let us examine how gene regulatory interactions can collectively give rise to a global network behavior that satisfies the requirements for development of a specialized cell phenotype, and eventually, a whole living organism. Without distinct regulatory interactions between the genes, any combination of gene activities across the genome

would be possible. This would result in an unstructured continuum of gene activation profiles, but no directed developmental processes, no robust expression profiles, and hence no differentiated cell types. In reality, genes interact with each other through the regulatory proteins they encode. Each gene (or its encoded protein) has a very specific set of interaction partners based on its molecular structure, and each interaction has a distinct mode (e.g., stimulatory, inhibitory, etc.). Thus, the genome contains a hard-wired interaction network. Interactions between genes therefore introduce constraints in the whole network, such that many gene activation profiles become unstable and are never realized. It is this collapse of the vast space of theoretically possible configurations of gene activity combinations that leads to distinct dynamics and the robustness of a limited number of cell phenotypes (Kauffman, 1993).

In technical terms, constraint of the dynamics by these molecular interactions means that the *high-dimensional state space* of gene activation is structured. One can define a state space as the *N*-dimensional space in which every point represents a different network state defined by a distinct gene activation profile, where *N* = number of genes. Now assume that gene A unconditionally inhibits the expression of gene B; then all network states in which both A and B are active will be unstable, thus forcing the network to “move” in state space until it hits a stable state. The network may also cycle between a few states. Thus, taking all the interactions into account, it can be shown that the network can change its activity profile in only a few directions (following stable trajectories) until it reaches a stable state, the so-called “*attractor state*”, which can be a fixed-point or cycling attractor (Kauffman, 1993). The existence of unstable regions and of multiple stable attractors impose a substructure to the state space, which

might be imagined as an "*attractor landscape*" as shown in Fig. 4. Accordingly, the state of the network (and hence of the cell) can be viewed as a marble on that landscape: it is forced to roll along valleys (trajectories) into the pits (attractors) (Fig 4.). This attractor landscape therefore captures the constrained, global dynamics of cell fate switching (i.e., phenotypic control). In fact, Waddington, Delbrück, Monod and Jacob and Kauffman have all proposed (in various forms) that the distinct, phenotypic differentiation states that we observe in living systems correspond to attractors in the state space defined by the molecular activities of the underlying network (Waddington, 1940; Delbrück, 1949; Monod and Jacob, 1961; Kauffman 1969). Thus, attractors in the state space map into to stable phenotypic states (differentiation to distinct cell types, cell proliferation, programmed cell death, etc.), and the trajectories represent directed developmental processes.

In the landscape of a real gene regulatory network, the attractors would represent cell states that are stable to many random perturbations. At the same time, the network would allow the cell to switch to other attractors given the appropriate sets of conditions, such as the presence of external regulatory signals that promote a particular cell fate (Huang, 2002). This highly structured landscape with latent, "preexisting" possibilities creates the stage on which the developmental program is played out. Interestingly, Waddington similarly proposed an "epigenetic landscape", with a marble whose position represents developmental state rolling down valleys (Fig. 5) based on his observation that cells "switch between distinct, well recognizable types" during development, and that intermediates are rare and unstable (Waddington, 1940; 1956). This picture captures the basic rules governing cell fate dynamics, and we can now argue that,

although proposed as an intuitive representation, Waddington's epigenetic landscape is in principle the state space of the molecular network that controls cell fates. Thus, the attractor landscape represents the emergent properties of the interaction network.

Much as the architecture of the cytoskeletal network affects the emerging macroscopic mechanical properties of cells and tissues, the architecture of the gene regulatory network (the wiring diagram of the gene-gene interaction) determines the specific "topography" of the attractor landscape, and hence cell behavior. However, not all network architectures give rise to a "reasonable" state space structure. For instance, using simulations of random continuous or discrete network models, it has been demonstrated that a fully connected network (in which every gene affects every other) would be unstable, that is, devoid of fixed-point attractor states. In contrast, sparsely connected networks are more likely to produce a dynamics with stable states (Kauffman, 1993; Glass and Hill, 1998). Apparently, the network architecture has been shaped through evolution by continuous growth and rewiring such that it gives rise to "biologically reasonable" dynamics with multiple stationary attractor states. These attractors are just stable enough to resist random perturbations, but at the same time, they allow the existence of multiple cell fates and switching between them in response to distinct perturbations during embryonic development.

The recent availability of information about large protein and gene networks (containing thousands of components) in baker's yeast (S. cerevisiae) made possible by new large-scale, high-throughput biochemical methods, has stimulated investigations into the natural architecture of biological regulatory networks. These studies revealed that the protein-interaction network and gene regulatory network are indeed sparsely

connected in these cells. Moreover, several interesting features of the network architecture, such as a near power-law distribution of connectivity (number of interaction partners per molecule), a propensity to modularity and use of hierarchical structure were all found to be present (Jeong et al., 2001; Wagner et al., 2002; Maslov and Sneppen, 2002). Interestingly, the (near) power-law architecture appears to have beneficial consequences for system-wide dynamics (Fox and Hill, 2001; Huang et al., in preparation). Specifically, the regime in the space of possible network architectures in which "biologically reasonable" networks (i.e., which exhibit ordered behavior with small attractors) is larger because the networks tolerate higher connectivity without becoming chaotic.

5.3. Biological implications of attractor states

As in the case of structural networks and the tensegrity model which allow the prediction of some macroscopic mechanical properties of the cell based on emergent features of the model, the generic global behavior of the cell is predicted by the model of an attractor landscape. In fact, the existence of distinct stable cell fates (proliferation, apoptosis, quiescence, etc.) and of different differentiated cell types (liver, skin, neuron, etc.) that are robust to perturbation, yet can switch between these same distinct states under restricted conditions, is itself a strong indication that there are attractors of an underlying molecular network. Similarly, robust developmental trajectories, corresponding to long valleys leading to lowest points in the landscape, can be explained as emergent properties of the genome-wide network of genetic interactions. However, the dynamic networks approach and attractor landscape formalism may also provide new insight into other cell biological phenomena that have previously resisted

straight-forward explanation by the conventional paradigm that emphasizes the role of individual signal transduction pathways.

Cell fate regulation in tissue homeostasis. As predicted by the dynamic network model, cell fates represent discrete, mutually exclusive, stable states that require specific signals to transition to each other, when such a transition is possible. For instance, differentiation and proliferation are well known to be mutually exclusive and robust (Goss, 1967) ; in many cell systems just quitting the proliferation state by overexpressing the cell cycle inhibitor protein p21 forces the cell to automatically enter the differentiation program (Steinman et al., 1994; Parker et al., 1995; Das et al., 2000) . That cell fates are robust and can be realized just by "placing" cells in the corresponding "basin of attraction", from which they will reach the attractor state, is best recapitulated by the observation that many non-specific pharmacological stimuli that activate multiple proteins across several signaling pathways often trigger expression of the same set of cellular phenotypes. For instance**,** differentiation of many cell types can be turned on by a large variety non-specific agents, including DMSO or ethanol (Yu et al., 1994; Spremulli et al., 1984, Messing et al., 1993; Bogomolova et al, 2001; Kulyk et al, 1996). Specifically, the differentiation of a promyelocyte cell line into mature neutrophils (the major white blood cells involved in innate immune response) can be elicited not only by DMSO, but also by treatment with retinoic acid, hypoxanthin, actinomycine D, flavone, etc (Collins, 1987). In these cases, it appears that simultaneous perturbation of multiple targets in different pathways results in the channeling of the biochemical effects into common end-programs, and hence the same "default" cell fate.

Perhaps the most striking cellular manifestation of the principle that cell fates represent attractor states comes form experiments in which cell shape was varied as an independent control parameter using microfabricated geometric islands of extracellular matrix proteins to which mammalian cells normally adhere (Chen et al., 1997; Huang et al., 1998). The traditional mechanistic, pathway-centered explanation of cell fate switching assumes that a specific, "instructive signal", i.e., a messenger molecule that interacts with its cognate cell surface receptor, tells the cell which particular genes to activate in order to establish a new cell phenotype. However, when these instructive signals (e.g., soluble growth factors and insoluble extracellular matrix molecules) were held constant, cell shape distortion alone was able to switch endothelial cells between proliferation, apoptosis and differentiation (Huang and Ingber, 2000). Thus, variation in one continuous control parameter (cell shape) that is devoid of the molecular specificity normally assumed to carry "instructive" information led to switching between multiple, mutually-exclusive cell fates, and produced effects reminiscent of a biological "phase transition". Essentially, cell distortion triggered the cell to "select" between different preexisting attractor states.

Integration of Structural and Information Networks. Importantly, because cell shape is governed by changes in cytoskeletal shape and mechanics, phenotypic control by cell distortion is a clear example of how structural networks can impact information processing networks in living cells. From a mechanistic point of view one can then ask, how can a "non-specific" parameter, such as cell shape elicit the detailed molecular changes associated with cell growth, differentiation and apoptosis? If cell fates are attractors, then a large variety of molecular signals will push the cells into the few

available behavioral modes that the cell can adopt: again, regulation corresponds to selection among a limited number of preexisting fates, rather than instruction of how to behave.

Changes in cell shape imposed by the microfabricated constraints lead to massive rearrangements of the cytoskeleton that maintains shape stability in response to external influences according to the tensegrity rules that govern these structural networks. Visualization of the actin cytoskeleton in cells grown on micropatterns, for example, revealed that the actin bundles of the cell reorient depending on the shape of the microfabricated adhesive island and map out tension field lines within the cell (Parker et al., 2002; Chen et al., 2003).

But how does cell mechanics affect cell fate? Cell anchoring to extracellular matrix substrates, such as these islands, is mediated by cell surface integrin receptor molecules that cluster within small anchoring sites known as “focal adhesions”. Actin filaments insert at these focal sites of attachment between integrins and the extracellular matrix, and apply traction forces to these adhesions much like the tension in a tent membrane is transmitted through ropes to the pegs that anchor it into the ground. These cellular anchoring structures at the cell membrane are also the nucleation sites for the formation of large, multimolecular complexes of proteins that are involved in signal transduction, and hence mediate cellular information processing (Geiger and Bershadsky, 2001). Such complexes at adhesion sites facilitate the interactions between signaling proteins. For instance, the activation of many of the signal transduction molecules, such as the aforementioned ras-raf-MEK-Erk mitogenic pathway depends on the configuration of the actin-cytoskeleton (Howe et al., 2002),

whereas assembly of signaling protein complexes at the focal depends on the tension in the actin bundles (Chrzanowska-Wodnicka and Burridge, 1996). Thus focal adhesions also represent sites of mechanotransduction (Ingber, 1991; Geiger and Bershadsky A 2002]: they sense the mechanical tension of the cell that is modulated in response to the geometry of the environment (Parker et al., 2002).

As described above, the 3D shape of molecules dictates their mechanical and biochemical behavior; another example of emergence from the level of their component parts (e.g., from amino acids to catalytic enzymes). Importantly, altering molecular shape through chemical modification or mechanical distortion alters biochemistry by changing thermodynamic and kinetic parameters (Ingber, 1997; Ingber, 2003b). The biochemical information processing network of the cell is therefore governed by physical interactions that depend on the 3D shape and mechanical properties of the individual molecules and hence, on the state of the cytoskeletal network that they comprise. Thus, structural networks and information networks integrate as a result of mechanochemistry. Specifically, mammalian cells contain structures that link cytoskeleton with signaling pathways, thereby allowing mechanical forces to feed back to regulate cellular information processing.

The biochemical details as to the precise molecules that transduce the mechanical forces into biochemical signaling are not still not fully understood, although strong experimental evidence now support the implication of several specific signal transducing proteins (Geiger and Bershadsky, 2001). However, given the fact that researchers commonly strive to uncover all of the “instructive pathways” by which cell fates are regulated, it appears that instead of being carried along linear molecular pathways,

information is processed in a distributed manner over the network of interacting regulatory molecules. Many of these molecules physically associate with the load-bearing elements of the structural cytoskeletal network that stabilizes cell shape. If the activities of associated regulatory molecules were to change in response to mechanical distortion, this integrated structural and information processing network would be perfectly designed to sense the diffuse signals that emanate from a concerted rearrangement of the cytoskeleton in response to mechanical stress or physical changes in cell shape. In fact, both molecules that physically associate with the cytoskeleton in the focal adhesion site and at other locations throughout the cell have been shown to change their activity in response to applied mechanical stress or cell distortion (Geiger and Bershadsky, 2001).

Because the wiring of the signaling networks produce attractors that correspond to only a limited number of distinct cell fates, the cell may naturally and reliably sense a broad spectrum of signals and simultaneously orchestrate multiple molecular responses to produce coherent behavioral programs. In other words, the existence of attractors representing distinct cell behaviors allows the evolution of a form of regulation that connects signals devoid of molecular specificity like mechanical forces to the internal regulatory machinery that governs specific cell fates.

5.4. Experimental evidence for attractors in gene regulatory networks.

The characteristic dynamics of cell fate control, the mutually-exclusivity of different phenotypes, and their robustness in living cells all suggest that distinct cell fates represent attractors that emerge in the dynamic network of gene regulatory interactions. But can we directly view the structure of the attractor landscape without knowledge of

the precise wiring diagram of the underlying genome-wide regulatory network at the molecular level? To map out this state space, it would be necessary to simultaneously measure the activation state of the genome-wide set of molecular activities that are responsible for cell fate switching. The arrival of technologies for the massively-parallel monitoring of genes now opens this possibility to follow trajectories of cell states in high-dimensional state space of the regulatory network. Gene expression profiling using DNA microarrays allows the parallel measurement of the level of >10,000 mRNAs in cells and tissues; this represents a surrogate measure for genome-wide gene activation profiles, and hence for cell states.

One way to uncover the existence of a high-dimensional attractor in real cells, where unlike in computer simulations we cannot systematically sample the state space, is to approach it from different directions of the state space and demonstrate the convergence of the trajectories (Fig. 4). Recently, we used genome-wide gene expression profiling to probe trajectories in state space with human HL60 cells that are induced to undergo a switch between a proliferative, promyelocytic, stem cell-like state to a fully differentiated neutrophil phenotype. The cells were induced to differentiate by two different stimuli – DMSO or retinoic acid (Collins, 1987). Using this approach, we showed that the transition to the neutrophil differentiated state carries the signature of an approach to an attractor state: the two trajectories initially diverged (because of the distinct pharmacology of the triggering agents) but then converged with respect to more than 50% of the thousands of dimensions of state space that were monitored (Huang et al., in review). Further analysis will have to clarify why the convergence was incomplete.

Most likely, the differentiated state of the neutrophil cells is not one point in state space, but a cloud in a flat bottom attractor.

Detailed analysis of the changes of individual genes show that many of the almost 2000 genes found to contribute to the convergence of the two trajectories in fact behaved in an opposite manner at the beginning (i.e., up- vs. down regulation), but converged to the same expression levels at the end of the differentiation process. This counterintuitive behavior defies all current attempts to assign a biological role to individual genes by focusing on individual gene regulatory cascades. Conversely, with an attractor landscape, convergence from disparate activation profiles is inevitable when the cells enter the same new attractor state (Fig. 4). Unfortunately, the manipulation of activation state of individual genes in living cells is still cumbersome compared to the situation in computer-simulated networks, such that systematic network perturbations that may reveal more detailed information about the structure of the attractor are still limited. Nevertheless, this experiment is a first small step towards the molecular characterization of Waddington's "epigenetic landscape" (Fig. 5) and an essential intermediate step towards our understanding of how the genome maps into the phenome.

5.5. Hierarchical considerations: signaling networks beyond the cell

Similar to the structural networks discussed above, information networks will extend beyond the limits of intracellular regulation. Cells in various states (attractors) signal to each other via physical cell-cell contacts, soluble cytokines and insoluble matrix scaffolds, thus forming an extracellular communication network. The dynamics of such "cellular networks" can also be viewed in a framework of state space concepts, with

stable behavioral modes that involve many cell types and their secreted products representing a coherent, robust physiological program of the tissue, such as inflammation, immune response, regeneration, development, toxicity, etc. These distinct "tissue fates" also exhibit properties of state space trajectories and attractors. For instance, immune system decisions between mutually exclusive, robust responses are common place, as in the Th1 / Th2 dichotomy in the T cell immune response (Santana and Rosenstein, 2003).

Biomedical research is only at the beginning of appreciating these higher level interactions as formal networks, because most of leading edge "systems biology" research is still carried out on single cell model organisms. But experiments in the near future that elucidate these cell-cell interaction networks will enable the next step in understanding biological regulation: marching up to a higher level of organization in the vertical hierarchy of integration that characterizes complex living organisms.

6. CONCLUSION

Tensegrity is a principle that ensures for structural stability within networks comprised of multiple structural components, and hence governs their self-assembly. Tensegrity is used at all size scales in the hierarchy of life, and it may have played an important role in the mechanism by which hierarchical self-assembly of inorganic components and small organic molecules led to the formation of living cells (Ingber, 2000). Use of the tensegrity principle by cells also provides an energy-efficient way to build macroscopic hierarchical structures using tiers of interconnected molecular networks (Chen and Ingber, 1999).

On the other hand, the emergence of attractor landscapes within sparsely connected information processing networks provides a mechanism for establishment of a limited number of stable network states that may have enabled evolution to harness a wide variety of environmental signals, including mechanical perturbation, for the regulation of cell fates. Thus, from the perspective of organismal biology, linking tensegrity-based structural networks and physical constraints to cell fate regulation is a central requirement for the evolution of organisms of increasing size that cannot rely solely on chemical interactions with their environment for control of their behavior. Living cells and tissues must deal with macroscopic physical phenomena such as mechanical forces, including tension, compression, shear, surface tension, and osmotic stresses. These physical signals can regulate specific modes of cell behavior controlled by molecular networks because of the link between structural networks and biochemical reactions (e.g., mechanochemistry on the cytoskeleton), and because of the existence of information processing networks that produce an attractor landscape with stable states.

In both complex cellular structural networks and information networks, simple properties emerge through the collective action of the parts that includes both mechanical and biochemical interactions. Hence the study of network properties helps to bridge the gap between microscopic biochemistry and macroscopic structure and behavior. Thus, elucidation of how simple, rule-governed behaviors (e.g., mechanical properties of cells and their behavioral control) emerge at higher levels of organization may eventually lead to a fuller understanding of the inner working of living organism

across many size scales. Although our work represents only a first step, hopefully, it opens up an entirely new avenue of investigation.

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FIGURE LEGENDS

Fig 1. Hierarchical levels of organization in complex living organisms.
At each level of the hierarchy, new entities and level-specifc rules that govern their behavior emerge from the interacions of the entities of the lower level. Thus, there is no "scale-invariance" as in fractals. Here we focus on the "emergence" at the cellular and tissue level of the characteristic mechanical properties (left) and features of cell fate behavior (right).

Fig. 2. Tensegrity model. A prestressed tensegrity structure composed of 6 compression-resistant struts (white struts) interconnected by 24 tension cables (black lines) on its periphery; this model also contains radial cables connecting the ends of the struts to the cell center (red lines). The theoretical tensegrity model of the cell is based on this architecture. In the cell model, the black lines correspond to viscoelastic actin cables, the red lines to viscoelastic intermediate filaments (of different time constants), and the white struts to rigid microtubules.

Fig 3. Emergent mechanical properties of the tensegrity model: simulation versus experimental data.
A. Cellular elastic (*G'*) and frictional (*G"*) moduli predicted by a computational tensegrity model (solid lines) versus data obtained from experiments with living cells (circles). Data in **A** and **B** that show the dependencies of moduli on prestress are re-plotted from Stamenovic et al., 2002; data in **C** and **D** that show frequency dependencies are re-plotted from Fabry et al, 2001. The frequency (ω) is given in Hz, whereas prestress and elastic and frictional moduli are in Pa.

Fig. 4. Cell fates as attractors.
The struture of the N-dimensional state space (*N* = number of interacting genes in the network, e.g. *N* = 10,000) is schematically shown as a three-dimensional topographic "attractor landscape" which is conceptually equivalent to Waddington's "epigenetic landacape" (Fig. 5). Each point in the landscape represents a cell state *S*, defined by

the profile of the activation state x (measured as mRNA level) of all the N genes: $S = [x_1(t), x_2(t),...x_N(t)]$. The pits in the landscape are the attractor states which represent the stable cell fates, in this case, the precursor cell and the differentiated neutrophil. Transition into the differentiated state can be triggered by two pharmacologically distinct differentiation-inducing agents which perturb the state of the precursor cell in different ways such that the cells takes two different trajectories, A and B, respectively, to reach the neutrophil state. Monitoring the change of $S(t)$ along these two trajectories, $S_A(t)$ and $S_B(t)$ respectively, as the change of gene expression profile using DNA microarrays allows the calculation of the inter-trajectory distance D (Huang et al., submitted). The inset on top shows the time course of D using for a subset of 2600 genes after DMSO and retinoic acid induced neutrophil differentiation. D was measured as 1- r, where r is the Pearson correlation coefficient between two profiles. The course of D shows initial, rapid divergence of the trajectory, followed by terminal convergence in more than 50% of the state dimensions as the cell reaches the differentiated state, indicating the approach to a high-dimensional attractor state.

Fig 5. Waddington's idea of "epigenetic landscape"

Although the model Waddington first proposed in 1940 to explain a cell's decision between distinct, "discrete" developmental fates was merely an intuitive metaphor, it may be regarded as the structure of the state space representing the dynamics of gene regulatory networks, the “attractor landscape” in Fig 4.