Chapter 2
Complexity and Information: Cancer as a Multi-Scale Complex Adaptive System

Parag Mallick

Life is a relationship among molecules and not a property of any molecule.

—Linus Pauling

Cancer is no more of a disease of cells than a traffic jam is a disease of cars. A lifetime of study of the internal combustion engine would not help anyone to understand our traffic problems. The causes of congestion can be many. A traffic jam is due to failure of the normal relationship between driven cars and their environment and can occur whether they themselves are running normally or not.

—D.W. Smithers, Lancet, March 1962 (Smithers 1962)

Introduction

Our current understanding of biology and cancer is an implicit model of cellular and organismic regulation with its roots in early biochemical genetics inquiries. The concept that a gene is responsible for a particular protein and can be responsible for a disease was first proposed in 1908 by Archibald Garrod, an English physician (Garrod 1908). Garrod was interested in heritable diseases containing "inborn errors of metabolism." He suggested (correctly) that alkaptonuria results from a single recessive gene, which causes a deficiency in the enzyme that normally breaks down alkapton. It is now known that alkaptonuria is caused by a defect in homogentisate 1,2-dioxygenase which impairs the degradation of tyrosine (La Du et al. 1958; Zatkova 2011). Beadle and Tatum's subsequent work demonstrated that single gene mutations could incapacitate specific enzymes, so that neurospora with these

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mutations had significantly altered physiology—they required an external supply of nutrients to generate something that endogenous enzymes normally produced (Beadle and Tatum 1941).

These results led them to the single-gene/single-enzyme hypothesis, which states that each gene is responsible for directing the construction of a single, specific enzyme. Many researchers, including Meyerhof (Meyerhof 1945; Meyerhof and Junowicz-Kocholaty 1943; Meyerhof and Oesper 1947), have contributed to advancing the concept of "enzymatic pathways" through the elucidation of glycolysis. Taken together, these studies suggested that aberrant physiology (i.e., disease) could readily occur through the alteration of one or several genes that had immediate implications in "pathways."

Through the worldview proposed by early biochemical geneticists, the relationship between genotype and phenotype was straightforward. Furthermore, the gene-centric approach was a robust, self-consistent model for biology and was able to readily explain a number of diseases and biological phenomena. A natural consequence of this single-gene/single-enzyme view of biology is that the major focus of cancer investigation has been identifying genes and gene products whose alteration leads to carcinogenesis or to changes in the "phenotype" of cancer cells. In this worldview—the phenotype is a sum of its parts, or "genotype."

The approach of determining the origins of phenotype by deconstructing the system into its component parts may have roots in much earlier thinking, like René Descartes, who posited that complex situations can be analyzed by reducing them to manageable pieces, examining each individually, and reassembling the whole from the behavior of the pieces. At the time, during the scientific revolution of the sixteenth and seventeenth centuries, this mathematical, positivistic perception was novel relative to the prevalent descriptive-metaphysical perceptions they replaced. Descartes' reductionism preceded biology and could not have anticipated many of the challenges that arise in complex systems. Reductionist investigations continue to drive present-day biology and lead to the simple assumption that higher levels in a biological hierarchy can easily be understood from the behavior of the lower levels.

Mechanistic biology also had its roots in the seventeenth century and was influenced by the same factors that led to reductionism. The successes in Newtonian physics and in clockworks led to the belief that everything, including organisms, was based on simple clockwork-like, easily understood, deterministic principles. Mechanistic biology was most formally expressed by Jacques Loeb in 1912. His book reflected the common view of the time. All biological behavior, he concluded, was predetermined, forced, and identical between all individuals of a particular species; organisms were thus merely complex machines. Although environmental transduction mechanisms were completely unknown, Loeb assumed they must be rigid, invariant, physico-chemical mechanisms like the cogs in a clock.

In much the same way that Newtonian physics explains a lot, but not all of the behavior of objects in motion, the early views of biological regulation fail to fully explain or predict the biology. The largest hole in early models is a failure to account for the impact of context. By applying formalisms from systems and complexity

theory we arrive at a very different view of the disease. We find that biology, in general, and cancer in particular can be viewed very naturally as a complex adaptive system (Deisboeck and Kresh 2006; Schwab and Pienta 1996). By altering our perception of cancer we may gain a deeper understanding of the disease, uncovering new ways to prevent it, diagnose it, and treat it.

Though systems-thinking can be traced back to early pre-Socratics of the sixth century B.C.E., it is clearly articulated in an Aristotelian world view, which focuses on the holistic as summarized in his statement "the whole is more than the sum of its parts." In modern times, systems approaches were significantly advanced in the late 1960s and 1970s by researchers such as Bertalanffy (1973; Von Bertalanffy 1972) and Laszlo (1972).

At a basic level, a system can be defined as a set of interacting, interdependent components. Systems theory provides a vocabulary and approach for modeling the behavior of any group of objects that work in concert to produce some result. Simple systems display superposition, scaling, and homogeneity, thus allowing one to readily explain behaviors driven purely by the components and not interactions amongst those components. However, interdependence is a critical feature of systems. Mathematically, if there were no interdependence and the result of a set of variables contained no cross-terms, by definition, the whole would be the sum of its parts.

A system is considered complex if it displays emergence and self-organization. In other words, if the behavior of the whole is difficult to predict from the behavior of its parts the system is complex (e.g., water formation). A complex system is adaptive if the agents as well as the system are adaptive. Systems (simple, complex, or adaptive) may be composed of other systems. Importantly, complicated and complex are not the same. There are many systems with numerous interacting parts (e.g., your laptop) whose behavior is not complex.

Typically, when studying complex systems we ask a set of questions:

- What are the components?
- What are the connections between components?
- What are the states of the components and the system as a whole?
- How do those states evolve and transition?
- What impacts the evolution of those states?
- What are the emergent behaviors?
- How does the system itself evolve?

Historically, much of systems biology has focused on the first two questions. However, there is a much wider set of questions affiliated with complex systems studies. Furthermore, complex adaptive systems display a variety of sophisticated properties, including:

Nonlinear behavior: The component parts do not act in linear ways. The superposition of the actions of the parts is not the output of the system. Small perturbations may lead to large effects (e.g., transitions in bi-stable systems).

Emergent behaviors: Properties are not obvious from the properties of the individual parts.

Self-organization: Order appears from the chaotic interactions of individuals and the rules they obey.

Adaptation (evolution): The environment becomes encoded in the rules governing the structure and/or behavior of the parts by a process of selection in which those that are better become more numerous than those that are not as fit.

Layers of description (nesting): A complex system may be composed of other complex systems. Additionally, a rule may apply at some higher levels of description but not at lower layers. Sometimes systems exhibit fractal scale-independent behavior and can be represented by the same models at different scales.

We use these properties as an organizing principle for this chapter showcasing diverse studies that provide examples of how these properties are widely prevalent throughout biology.

Research

Genome-Scale Models of Cellular Regulation: Nonlinearity

The torrential flood of data generated by -omics technologies has given us a fine-grained, detailed view of the world of genes, biomolecules, and cells—drowning us with data of immense complexity that we are just barely beginning to understand. Unfortunately, there is a deep chasm separating our knowledge of the molecular components of a cell and observations of cellular and organismic physiology—how these components interact and function together to enable cells to sense and respond to their environment, and to determine actions such as proliferation, migration, and apoptosis.

We do not understand on a fundamental level how information is transferred and processed in a biological system. Through mysterious processes, cells are able to take signals from their environment, process those signals, and then act. Unfolding this mystery of information transfer in biological systems is a critical challenge to modern biology. To unfold this mystery, physical sciences researchers attempt to develop models of information transfer and communication. Importantly, these communication systems have been shaped by millions of years of evolution and are additionally shaped by evolutionary forces within tumors. This extensive history makes it extremely difficult to develop effective and accurate models of cellular behavior.

Models of cellular regulation range from qualitative to quantitative, or from the conceptual to the mathematical. Biologists typically formulate their hypotheses (or models) in intuitive and conceptual ways, often through comparison amongst well-known systems. These biological models can be transformed into more quantitative models. In physics, mathematics is employed to describe physical phenomena. Similar approaches are required in biology to develop mechanistic and kinetic models of cellular phenomena.

Much of modern systems biology has focused on elucidating the components of a cell (which transcripts are present and in what abundance) and their connections to each other (which proteins interact or are co-regulated). These studies have led to increasingly complicated models of cellular regulation. These models often contain thousands or tens of thousands of components and are fundamentally rooted in the concept of "pathway." There are currently hundreds of molecular interaction and pathway databases. In theory, these resources should enable building or validating models of how cells use their component machinery to achieve homeostasis and response; however, there is a significant lack of compact, principle-based models illustrating the ways in which biology self-regulates.

Much like an architectural model is a replica of a building, models of cellular regulation are meant to be *in silico* replicas of the system. A biologic model conjoins a set of assumptions and declarations to reproduce or illustrate the behavior of a system and, importantly, to offer predictions for testing the model's validity. A clear definition of the system is the required first step in modeling. For example, the system of earth, its moon, and the sun is complicated. It is potentially very complicated if one includes details such as the composition of the earth and its atmosphere, as well as details about each crater on the moon. However, if the aim of the modeling is to plot the trajectories of earth around the sun and the moon around the earth, then it is sufficient to model the earth, sun, and moon as point masses and use Newton's universal law of gravitation to calculate the trajectories from aphelion to perihelion. Sometimes such compressions are not possible, i.e., there are no details that can be abstracted away. All of the details available are necessary to accurately describe the behavior of the system.

One of the key initiatives of our study was to examine work involving very compact models of regulatory mechanisms. This work has attempted to uncover fundamental properties of biological systems, asking why they are designed (or have evolved) to operate the way that they do and how it is that they are able to display non-linearity—a critical feature of biological regulation.

Dr. Jens Timmer of the Freiburg Institute, Germany, (site report, Appendix B) is attempting to uncover the general principles governing regulatory processes (Bachmann et al. 2012; Becker et al. 2010, 2012). In one study, he looked at a bacterial signaling network to investigate the impact that diverse topologies might have on its function (Kollmann et al. 2005). In particular, he asserted that a network should have the following properties: (1) It should be robust to noise, stable under cell-to-cell fluctuations of protein concentration by factor of 10, have the ability to sense and respond to relative changes of attractant concentrations as small as 2 % over a dynamic concentration range of five orders of magnitude and precise adaptation; and (2) It should be able to return to the same level of pathway activity under conditions of continuous stimulation. Given these design constraints, Timmer evaluated a range of topologies for the impact they might have on regulatory behavior determining what the necessary complexity might be and the source of the non-linear regulation. He also has expanded this work to other systems, including cytokines. These principles of network design are likely to help interpret a wide variety of systems with larger numbers of components. Notably, even this compact model, which did not

contain the rest of the regulatory circuitry, was able to match experimental data. Additional work in the role of noise in biological regulation is actively ongoing in the El-Samad lab (Stewart-Orstein et al. 2012). Dr. van Oudenaarden discusses the issue of noise in biological systems extensively in two of his recent publications (Munsky et al. 2012; Balázsi et al. 2011).

Work at Kyoto University, Japan, directly explored nonlinear processes in biological systems in their Laboratory of Bioimaging and Cell Signaling directed by Dr. Michiyuki Matsuda. The lab is focused on visualizing the growth signal transduction cascades in living cells. In general, there is a significant focus on dynamic living systems based on multi-dimensional quantitative imaging and mathematical modeling. The program involves investigators from a number of departments (graduate schools of Medicine, Biostudies, and Informatics; Institute for Frontier Medical Sciences; Institute for Virus Research; and Imaging Platform for Spatio-Temporal Information). They developed a unique FRET-based pipeline that measures key parameters associated with protein signaling (Aoki et al. 2008, 2011). They were able to measure most, if not all, of the kinetic parameters required for kinetic simulation of the MAPK/ERK signaling pathway, using HeLa cells as a model system. The model requires four classes of parameters: protein concentrations, association/dissociation rates, nuclear import/export rates, and phosphorylation/dephosphorylation rates. They experimentally determined approximately 30 parameters. Through a combination of this rigorous experimentation coupled with kinetic modeling they were able to show complex non-linear dynamics in signaling systems. They also showed how molecular crowding could further complicate signaling (Aoki et al. 2013).

At Hong Kong's Institute of Computational and Theoretical Studies, Dr. Lei-han Tang is investigating nonlinearity and noise in gene expression. In recent work, their group developed a model of transcription that includes three processes: transcriptional bursting in the nucleus, mRNA transport, and mRNA decay in the cytoplasm (Xiong et al. 2010). They generally observed that the extent of burst attenuation is governed by the rate of transport. The slower the mRNA transport, the smaller the noise in the cytoplasmic mRNA number. In the case of the Michaelis-Menten transport, the saturation effect of transport mediators or nuclear pores further reduces mRNA copy number fluctuations in the cytoplasm allowing for a dampening of the noise. In the context of gene expression in eukaryotic cells, their results indicate that transcriptional bursting can be substantially attenuated by the transport of mRNA from nucleus to cytoplasm. Saturation of the transport mediators or nuclear pores contributes further to the noise reduction.

A common thread in biological models has been the role of cooperativity in DNA structure as a control element. Dr. José Vilar from the University of the Basque Country, Spain, (site report, Appendix B) highlighted two examples of DNA proximity leading to nonlinear regulatory effects (Fig. 2.1). In one example, Dr. Vilar presented early work on the lac repressor (Vilar et al. 2003; Vilar and Leibler 2003), which binds to a primary operator O1 and prevents the RNA polymerase from transcribing the genes (Fig. 2.2). If it is not bound, transcription proceeds at a given rate. In addition to O1, there are two sites outside the control region, the

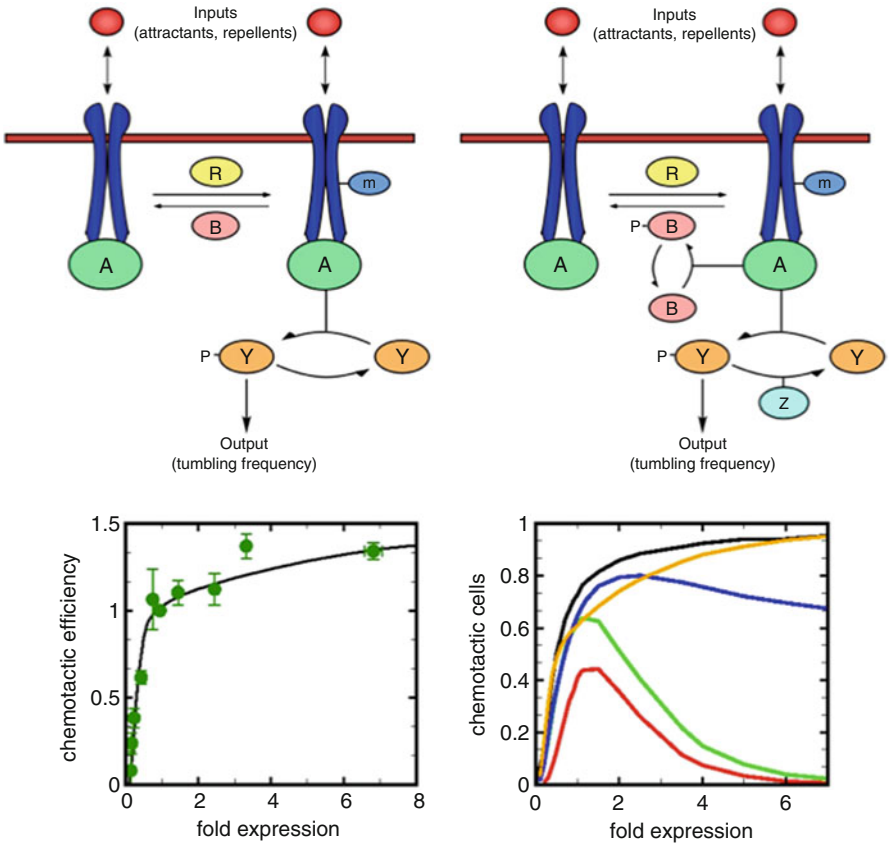

Fig. 2.1 Two possible topologies of a regulatory network (From Kollmann et al. 2005)

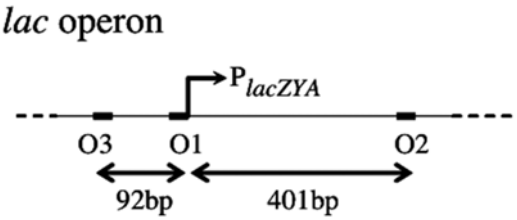

Fig. 2.2 The structure of the *lac* operon (From Vilar and Leibler 2003)

so-called auxiliary operators O2 and O3, which closely resemble O1 and can also bind with the repressor. However, they are much weaker than O1 (10 and 300 times weaker). Moreover, elimination of either one of them leaves the repression level practically unchanged. However, the role of O2 and O3 are actually quite significant: simultaneous elimination of both of these operators reduced the repression level about 100 times. Deeper investigations and computational modeling were able to detail how DNA looping could explain this result (Saiz and Vilar 2007). Dr. Vilar

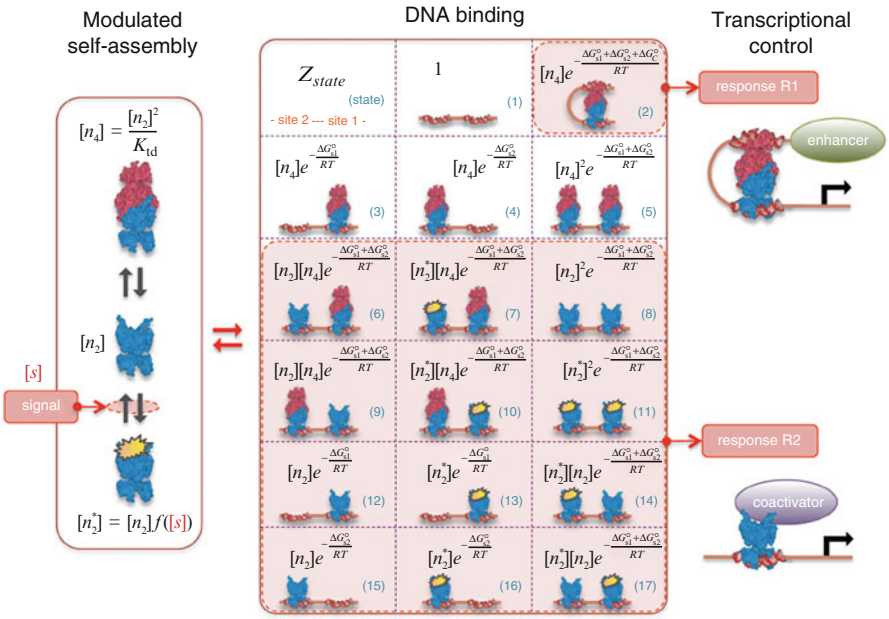

Fig. 2.3 Quantitative modeling of control gene expression by modulated self-assembly of the retinoid X receptor (RXR) (From Vilar and Saiz 2011)
The model considers how intracellular signals are processed through modulated self-assembly into populations of different RXR oligomeric species that upon DNA binding engage in transcriptional control

then proceeded to demonstrate how general processes of self-assembly could lead to these sorts of emergent behaviors in regulation by mechanisms previously thought to be distinct, such as in retinoid X receptor regulation (Fig. 2.3; Vilar and Saiz 2011).

Dr. Vilar's gene-scale findings are recapitulated at the chromosomal scale by Dr. Marc Marti-Renom of the National Center for Genomic Analysis in Barcelona, Spain (site report, Appendix B) (Bau and Marti-Renom 2012; Marti-Renom and Mirny 2011; Sanyal et al. 2011; Umbarger et al. 2011). This work parallels the work of Drs. Michor (De and Michor 2011) and Mirny (Fudenberg et al. 2011) supported by the National Cancer Institute Physical Sciences-Oncology Center (NCI PS-OC) program in the United States (Fig. 2.4).

Techniques from structural biology can be used to reconstruct chromosomal structure and demonstrate how long-range interactions may play a role in regulation. Dr. Helmut Schiessel at the Instituut-Lorentz, Netherlands, (site report, Appendix B) also identified many examples of genome structure playing a role in cellular regulation (Prinsen and Schiessel 2010). His group showed how the wrapping and unwrapping of the nucleosome allowed regulatory DNA binding sites to become exposed (Fig. 2.5).

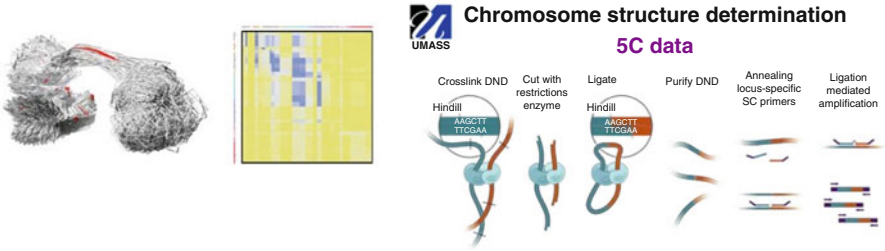

Fig. 2.4 Two computational approaches for determining the 3D structure of genomic domains and genomes (From Marti-Renom and Mirny 2011)

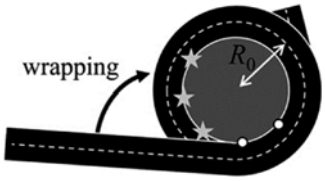

Fig. 2.5 A partially unwrapped nucleosome with exposed nucleosomal binding sites (*stars*). The nucleosome can lower its energy by closing those binding sites at the cost of bending the DNA (From Prinsen and Schiessel 2010)

Dynamical Systems, Cell States, and State Transitions

Dynamical systems models attempt to describe the temporal evolution of a system. To create a dynamical system we need to identify the "something" that will evolve and the rules describing that evolution.

In order to identify the "something," we need to come up with a set of variables that give a compact description of the system at any particular time. The variables do not have to fully describe a real-life system. However, the more complete the model, the more likely it will be able to accurately predict the system's behavior. It is assumed that by knowing the values of these variables at a particular time, we can accurately predict the state of the system at a future time. To model a real-life system, the modeler must decide what variables will form the complete description for the mathematical model. The variables used to describe the state of the dynamical system are called the state variables. The "state space" is the set of all possible states of the dynamical system; each state of the system corresponds to a unique point in the state space. The axes of the space are defined by the state variables. The state space may be of infinite size.

The second step in creating a dynamical system is to specify the rule for the time evolution of the dynamical system. This rule must be defined such that one can use current values of the state variables in combination with the rule to infer all future states. If the time evolution depends on a variable not included in the state space,

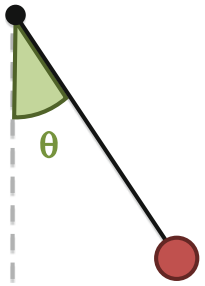

Fig. 2.6 A pendulum example can be used to explain a dynamical system (Courtesy of Parag Mallick)

then the rule combined with the state space does not specify a dynamical system. One must either change the rule or augment the state space by the necessary variables to form a dynamical system.

To make this more concrete, consider the example of a pendulum. The angle θ completely specifies the position of the pendulum (Fig. 2.6). However, we cannot use θ as the only state variable. If the above picture of the pendulum were a snapshot of a pendulum, we would not have enough information to know where the pendulum will move next.

Determining the future behavior of the pendulum requires knowing not only its position, but also its angular velocity. Therefore, the state space is the set of all possible pairs (angle, velocity). For this idealized pendulum, the angle θ and the angular velocity ω completely determine the state of the system.

Both θ and ω will evolve over time, and their value at one time determines all their future values. The dynamical system is 2D, and since θ and ω evolve continuously, it is a continuous dynamical system.

The dynamical systems approach is highly appropriate in biology. In biology we frequently refer to cells as having specific phenotypes, which may be analogized as states. For example, a cell may inhabit states such as dividing, apoptosing, and migrating. Accordingly, cells may have particular likelihoods of inhabiting particular states and of transitioning between states (e.g., metastatic potential).

Though significant effort has been made to determine diverse cellular phenotypes and to understand how endogenous and exogenous perturbations (e.g., mutations) lead to transitions in those phenotypes, our current approaches typically generate state spaces of very high dimension wherein each gene or protein in a cell might be considered as components of state.

During our survey abroad, we saw several exciting examples of dynamical systems approaches. For example, in Leipzig, Germany, the study team met Dr. Adalinde Uhrmacher from the University of Rostock (site report, Appendix B). Dr. Uhrmacher, a computer scientist, emphasized the importance of both modeling and simulation. Her group has designed a general purpose plug-in-based modeling and simulation framework that has already been applied to develop different modeling and simulation tools for cell biology (Ewald et al. 2010). Currently, the framework includes more than 700 plug-ins and more than 100 plugin types (e.g., different modeling formalisms, execution algorithms, steady state analyzers). It also provides intelligent support to configure suitable experiments on demand.

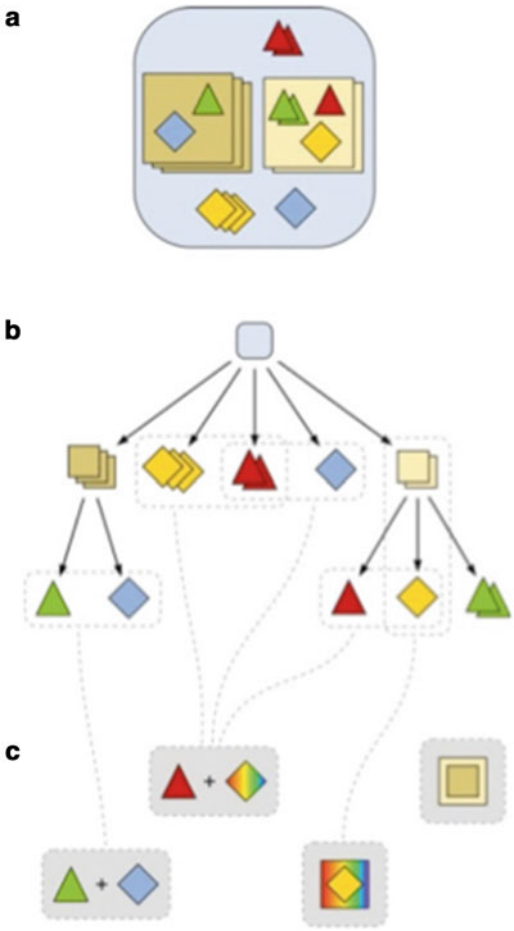

Fig. 2.7 Nested model structure (From Maus et al. 2011)
The hierarchical modeling concept. Different-shaped nodes correspond to different species while attributes are color-coded. Stacking of identical nodes represents the amount of a certain species. (**a**) A graphical representation of a hierarchical model structure via nested nodes. (**b**) The same model structure alternatively depicted as a directed tree graph. Note that in addition to atomic species (*triangles* and *diamonds*), species containing a sub-solution (*squares*) might be attributed so that each species at each level might have its own state. (**c**) Examples of matching different reactant patterns within the hierarchical model structure. The rainbow shadings in the second pattern (*diamond*) and third pattern (*rectangle*) illustrate variable instead of defined colors, i.e., attributes

Dr. Uhrmacher's general approach relies upon ML-Rules—a multilevel rule-based modeling method (Maus et al. 2011, Fig. 2.7), and a spatial variant—ML-Space (Bittig et al. 2011, Fig. 2.8). ML-Rules allows users to compactly describe and combine compartmentalized dynamics, including inter- and intracellular dynamics and processes at the cellular level such as proliferation of cells, apoptosis, and cell differentiation (Mazemondet et al. 2011). ML-Rules assumes

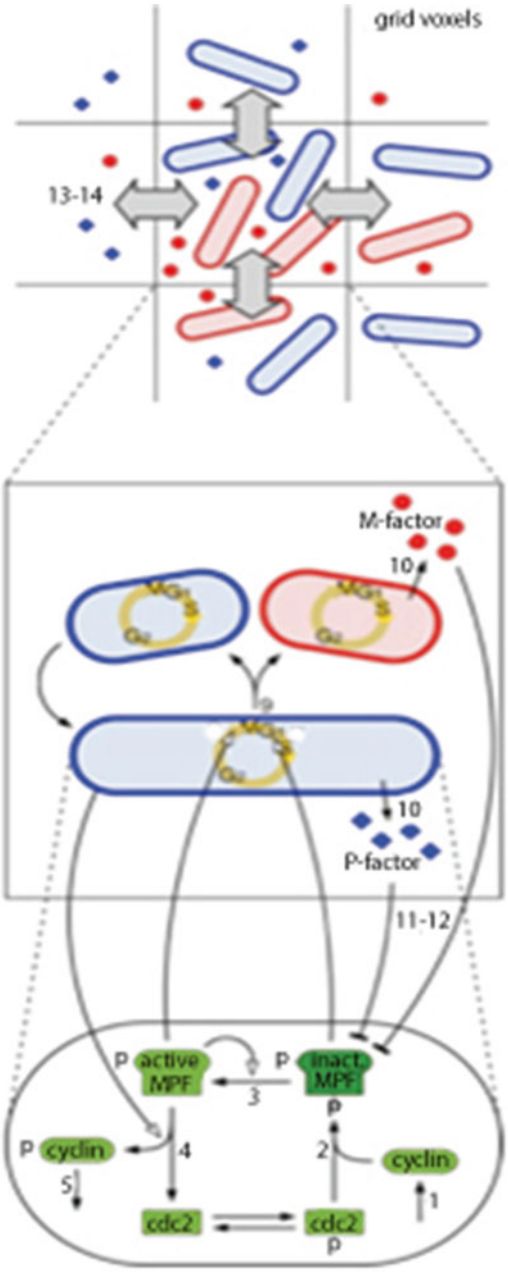

Fig. 2.8 Schematic description of the example model (From Bittig et al. 2011)
The model comprises three distinct hierarchical levels. At the *bottom level*, interacting proteins describe the intracellular dynamics of a fission yeast cell (reactions 1–5). The intermediate level describes dynamics of entire cell states, i.e., cell growth (6), cell cycle phase transitions (7–9), and division including mating type switching (9). In addition, cells may secrete pheromone molecules

well-mixed solutions within the compartments. It does not capture phenomena that are induced by the molecular crowding within cells. Therefore, the language for ML-Space has been developed with decidedly spatial semantics. Here, species can be defined as individual particles that react due to collisions, or as a population of species residing in a small area. It inherits from ML-Rules the compact description and the ability to describe processes at different organizational levels however they adhere to spatial physical constraints. ML-Space has been used to investigate lipid rafts as compartments, with a focus on their movements and the activity of receptors in rafts.

Dr. Hauke Busch of the Freiburg Institute for Advanced Studies, Germany (site report, Appendix B) presented his work, which is built upon the work done in the United States by Drs. Huang and Ingber (2006, Fig. 2.9) to characterize cell states and fate decisions. In this work (Busch et al. 2008) Dr. Busch asserted that the long-term phenotypic response of a cell can be expressed in terms of its slowest evolving functional elements. Postulating that a cell reaches a decision on a timescale of hours, its phenotype should be controlled by the slow protein turnover rates. To validate this finding they looked at the impact of hepatocyte growth factor (HGF) stimulation on keratinocyte cell migration.

Similar approaches are being investigated by Dr. Ping Ao at Shanghai Jiao Tong University. He hypothesizes (Ao et al. 2008; Wang et al. 2012) that in order to maintain the normal physiological function and developmental process for tissue specific function shaped by evolution, a minimal set of fundamental functional modules or pathways (e.g., cell cycle, cell death, inflammation, metabolism, cell adhesion, and angiogenesis) are needed. Each module can accomplish a relatively autonomous function, and cross-talk between modules allows one function to influence another. At the molecular-cellular level, it is hypothesized that the functional modules are deeply hierarchical and may be specified by important molecular and cellular agents, such as oncogenes, suppressor genes, and related growth factors, hormones, cytokines, etc. The interactions among these agents form an autonomous, nonlinear, stochastic, and collective dynamical network. The endogenous network may generate many locally stable states with obvious or non-obvious biological function. Normal state and cancer state are assumed to be stable states of the endogenous molecular- cellular network. The endogenous network may stay in each stable state for a considerably long time, and in certain conditions, stable states can switch between each other. In this hypothesis, the genesis and progression of cancer can be regarded as transition of the endogenous molecular-cellular network from normal stable state to cancer state (Fig. 2.10).

Fig. 2.8 (continued) (P-factor and M-factor) to the extracellular medium (10). Various inter-level causalities between the intermediate and the *bottom level* influence processes both in an upward (7–9) and downward causation manner (4, 11–12). The *top level* discretizes the environment of cells into multiple fictive compartments in order to study spatial dynamics of pheromone diffusion and displacement of cells (13–14). Note that, although spatial dynamics referring to compartments and particle diffusion between cells can be modeled, excluded volume effects cannot be described in ML-Rules therefore one has to move to ML-Space

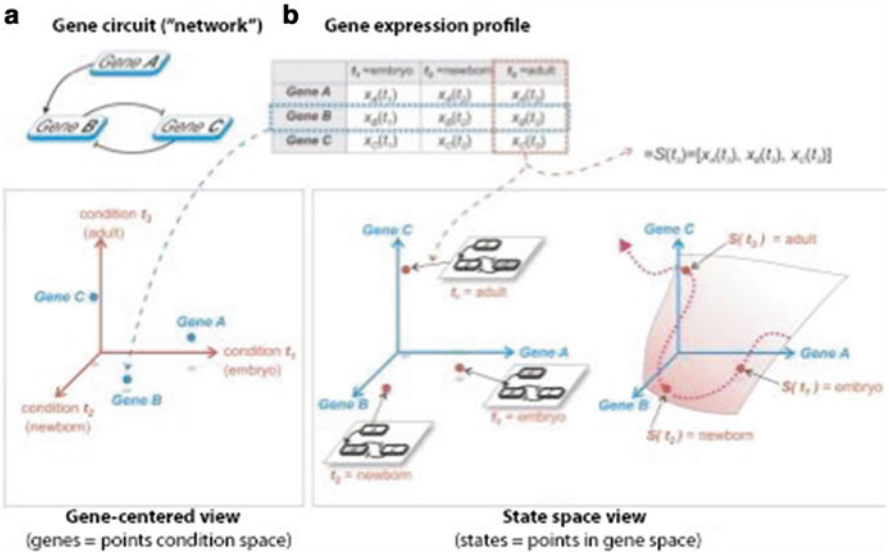

Fig. 2.9 The long-term phenotypic response of a cell can be described as a state space (From Huang and Ingber 2006–2007)

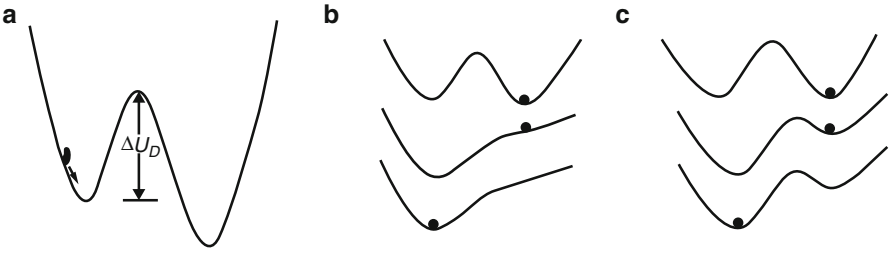

Fig. 2.10 Schematic diagram of the adaptive landscape of the phage lambda genetic switch, where the dynamic state of the biological system is represented as a *black dot* (From Wang et al. 2012) As the system progresses through different configurations, the dynamic state of the system may change or remain in a local minimum of the adaptive landscape. For full details of the endogenous molecular-cellular network hypothesis and its applications, see Wang et al. (2012)

State transitions and fate determination is hugely important in understanding therapeutic efficacy. At The Center for Quantitative Systems Biology in Hong Kong, Dr. Jue Shi is exploring cell-type variation in anticancer drug response (Chen et al. 2013). By combining experimental measurements of cellular alterations induced by drug treatment with kinetic modeling of pathway dynamics, the ultimate goal is to understand how different anticancer drugs perturb cellular behaviors and what variables as well as interaction modules in cellular pathways are the determining factors in engendering distinct drug response phenotypes in different cell types. In particular, they identified a novel, bimodal switch of p53 dynamics modulated by

DNA-damage strength that is crucial for cell-fate control. After low DNA damage, p53 underwent periodic pulsing and cells entered cell-cycle arrest. After high DNA damage, p53 underwent a strong monotonic increase and cells activated apoptosis. Their findings not only uncover a new mode of regulation for p53 dynamics and cell fate, but also suggest that p53 oscillation may function as a suppressor, maintaining a low level of p53 induction and pro-apoptotic activities so as to render cell-cycle arrest that allows damage repair.

Related studies are under way by Dr. Mariko Okada-Hatakeyama at the RIKEN Laboratory for Cellular Systems Modeling, Japan. The lab is investigating how activation of ErbB receptors by epidermal growth factor (EGF) or heregulin (HRG) determines cell fate decisions using a mix of experimental and computational approaches (Nakakuki et al. 2010). Although signals propagate through shared pathways, HRG and EGF generate distinct, all-or-none responses of the phosphorylated transcription factor c-Fos. In the cytosol, EGF induces transient and HRG induces sustained extracellular-signal-regulated kinase (ERK) activation. In the nucleus, however, ERK activity and *c-fos* mRNA expression are transient for both ligands. Knockdown of dual-specificity phosphatases extends HRG-stimulated nuclear ERK activation, but not *c-fos* mRNA expression, implying the existence of an HRG-induced repressor of *c-fos* transcription. Further experiments confirmed that this repressor is mainly induced by HRG, but not EGF, and requires new protein synthesis. Dr. Okada-Hatakeyama shows how a spatially distributed, signaling-transcription cascade robustly discriminates between transient and sustained ERK activities at the c-Fos system level. The proposed control mechanisms are general and operate in different cell types, stimulated by various ligands (Fig. 2.11).

An experimental approach to fate characterization was taken by Dr. Matthias Lutolf at the École Polytechnique Fédérale de Lausanne, Switzerland, (site report, Appendix B) who is attempting to control cell fates through microenvironment. He has developed 2D microwells (Gobaa et al. 2011) molded in hydrogel (Fig. 2.12 and see also Chap. 3). A major focus of his group's research is on the neural stem cell niche, in which he has shown that notch, jagged, and dll4 are involved in self-renewal of stem cells in his devices. Operationally, he is experimentally defining cellular state spaces for diverse cell types.

Self-Organization and Emergence

As noted above, complex systems are characterized to display emergent behaviors and self-organization. These properties are prevalent throughout chemistry and biology. For example, spontaneous collective motion can be observed in flocks of birds and schools of fish. One of the greatest mysteries in cancer biology arises from the observation that small length-scale perturbations (e.g., gene mutations) can lead to significant large length-scale effects (e.g., death).

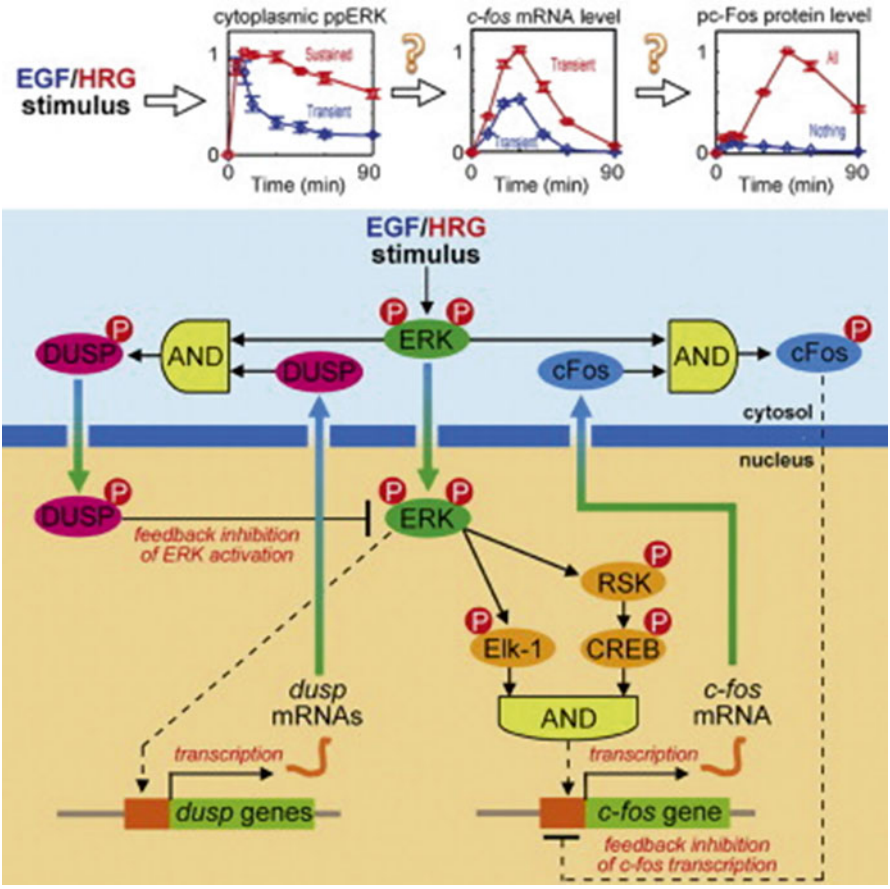

Fig. 2.11 Activation of ErbB receptors by epidermal growth factor (EGF) or heregulin (HRG) determines distinct cell-fate decisions, although signals propagate through shared pathways (From Nakakuki et al. 2010)
In the cytosol, EGF induces transient and HRG induces sustained ERK activation. In the nucleus, however, ERK activity and c-fos mRNA expression are transient for both ligands. Knockdown of dual-specificity phosphatases extends HRG-stimulated nuclear ERK activation, but not c-fos mRNA expression, implying the existence of a HRG-induced repressor of c-fos transcription. Further experiments confirmed that this repressor is mainly induced by HRG, but not EGF, and requires new protein synthesis

In looking at self-organization and emergence, we typically ask questions such as:

- How do collections of entities behave differently than either entity alone?
- What properties emerge from aggregate behavior?
- What information is communicated to aid in that self-organization?
- How is that information transduced?

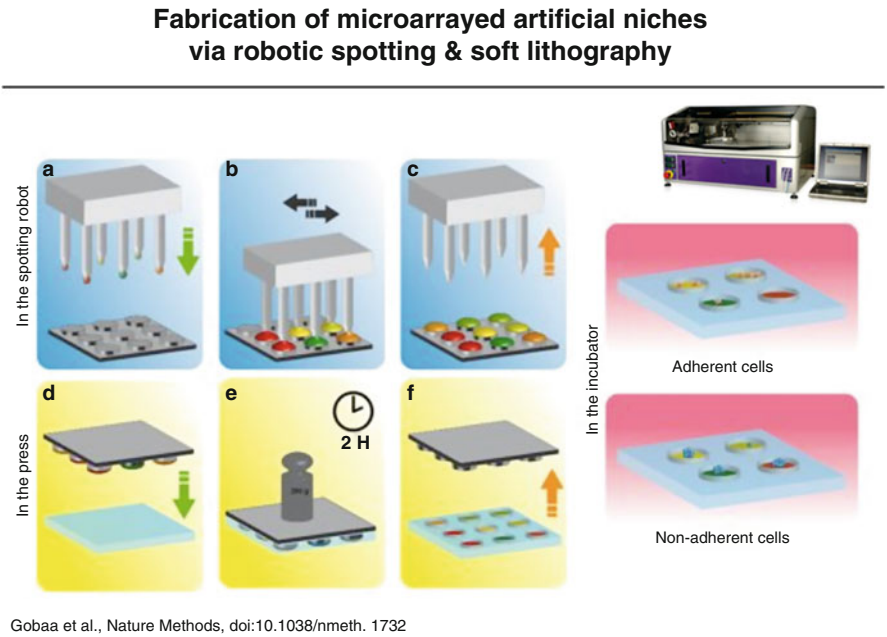

Fig. 2.12 A 2D microwell molded in hydrogel (From Gobaa et al. 2011)

Several groups are now actively pursuing this area at multiple scales.

At the molecular scale, Dr. Andreas Bausch's group at the Technical University of Munich, Germany (site report, Appendix B) has been actively engaged in studying the dynamics of actin assembly (Kohler et al. 2011; Schaller and Bausch 2012; Schaller et al. 2010). They have shown the emergence of collective motion in an actin/myosin motility assay. Motility assays, in which protein filaments are densely placed on a planar substrate, can show collective motion for high densities of motors and attached filaments. Notably, this motion is density dependent. At low density, fibrils have near random motion. However, above a threshold density, the filaments self-organize to form diverse moving structures such as swirls and interconnected bands (Fig. 2.13). These polar nematic structures are long-lived and can span length scales that are orders of magnitudes larger than their constituents. Recent work in Japan (Sumino et al. 2012) showed a similar pattern for microtubules.

At a cellular scale, the group led by Dr. Xavier Trepat at the University of Barcelona, Spain (site report, Appendix B) has focused on defining how cell and tissue dynamics are integrated to drive function. In particular, his group is one of the leaders in the emerging field of plithotaxis—the mechanism of innately collective cell guidance (Trepat and Fredberg 2011). To study this process, Trepat and colleagues have created a novel technique, monolayer stress microscopy, to characterize the local state of stress within a monolayer (Tambe et al. 2011). The technology allows the measurement of stresses within and between cells comprising a mono-

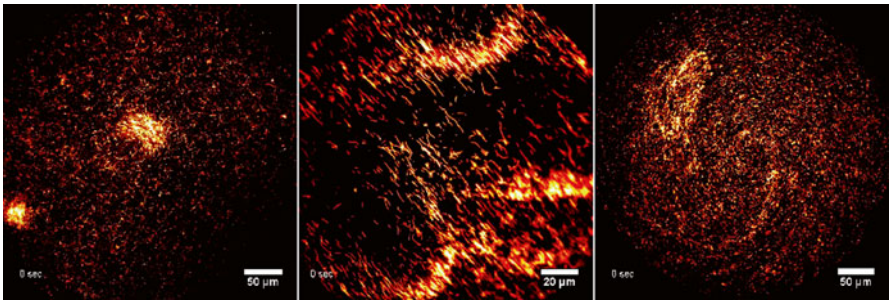

Fig. 2.13 An actin/myosin motility assay that shows that the motion is density dependent (From Schaller et al. 2010)

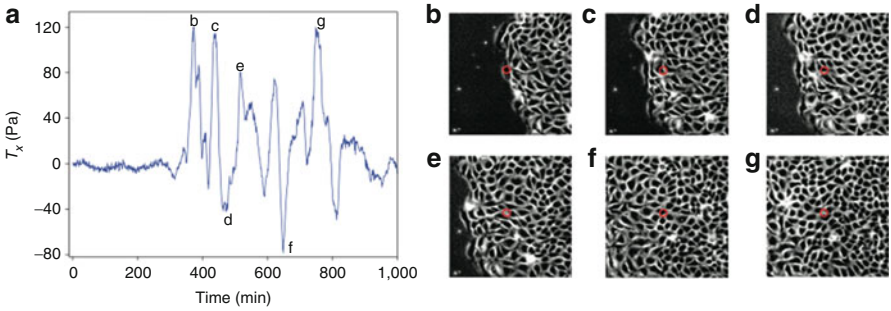

Fig. 2.14 High-resolution maps of stress components within an advancing monolayer sheet of cells (From Trepat and Fredberg 2011)

layer for the first time. Monolayer stress microscopy can generate high-resolution maps of stress components within an advancing monolayer sheet of cells (Trepat and Fredberg 2011). This work (Fig. 2.14) demonstrated that as cells in a monolayer expand, they do so "center-out," thus generating sinusoidal force patterns (Trepat et al. 2009). Though cells might migrate and grow in a number of different ways (e.g., front plane driven, uniformly, etc.), Trepat's technique was able to uncover a novel pulsing mechanism. Similar emergent multiscale phenomena are also being pursued by Dr. Yasuhiro Inoue at Kyoto University, Japan (Okuda et al. 2013a, b).

Tumor Evolution and Heterogeneity

Tumors, as collections of cells, are complex systems. They can be viewed from an evolutionary perspective as collections of cells that accumulate genetic and epigenetic changes, which are then evaluated relative to the selective pressures prevalent within an environment. Beneficial heritable changes can cause rapid expansion of

the mutant clone since they enable their carriers to outcompete cells that have not accumulated similar improvements. Mutations advantageous to the cancer cell are normally detrimental to the organism, ultimately causing death of both the patient and the tumor. Evolution generally selects for increased proliferation, survival, and evolvability on the cellular level, which leads to organ-scale consequences of progression, invasion, and resistance.

Investigations of evolution have been ongoing for hundreds of years—pre-dating Darwin. Physical sciences approaches combined with recent advances in genomic technologies have led to a renewed emphasis on cancer evolution. Work done in the United States by Dr. Rong Fan has shown that it is now possible to look at evolutionary processes with single-cell resolution (Fan et al. 2011). Furthermore, recent work from the United Kingdom has shown the extensive heterogeneity prevalent in cancers (Gerlinger et al. 2012). Evolutionary studies have analyzed the full spectrum of cancer from initiation through acquisition and penetrance of resistance.

With support from the NCI PS-OC program in the United States, Dr. Franziska Michor (Dana-Farber Cancer Institute; Chmielecki et al. 2011; Pao and Chmielecki 2010), and Dr. Robert A. Gatenby (H. Lee Moffitt Cancer Center & Research Institute; Gillies et al. 2012) have been leading evolutionary studies with a variety of stochastic models that rely on quantifying selective advantage. Other efforts, such as those of Dr. Carlo C. Maley (Greaves and Maley 2012), have focused on using evolutionary ecology approaches.

One approach employed by Dr. Oskar Hallatschek, from the Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany (site report, Appendix B) described how evolution begins with colony growth and proceeds to whole tumor growth. He is interested in range expansions—the movement of populations to different areas where they evolve separately. In his philosophy, there is typically a competition between Darwinian selection and genetic drift to drive evolutionary change. Genetic drift can have significant effects on small populations that may even lead to speciation. This is contrasted with large populations, where genetic drift is considered weak. Notably, in cancer, this general principle is violated when large populations undergo range expansions. The descendents of individuals first settling in a new territory are most likely to dominate the gene pool as the expansion progresses. Random sampling effects among these pioneers results in genetic drift that can have profound consequences on the diversity of the expanding population.

In this project, Hallatschek used simple microbial systems (Hallatschek et al. 2007) to study the nature of these number fluctuations (genetic drift) in range expansions of large populations.

This finding was first validated in bacteria (Fig. 2.15) with Dr. David Nelson, but has since been adapted to colon cancer and clonal expansion in neoplastic tissues (Martens et al. 2011). In these cases, the work allows for mutations to come in that confer a certain growth rate advantage. This model may be good for understanding the growth of intestinal epithelial cells out of the crypt (Fig. 2.16).

The lab of Dr. Jian-Dong Huang at Hong Kong Baptist University is taking advantage of evolution and fitness in a novel treatment strategy (Yu et al. 2012). Specifically, they take a synthetic biology approach to create a novel tumor-targeting

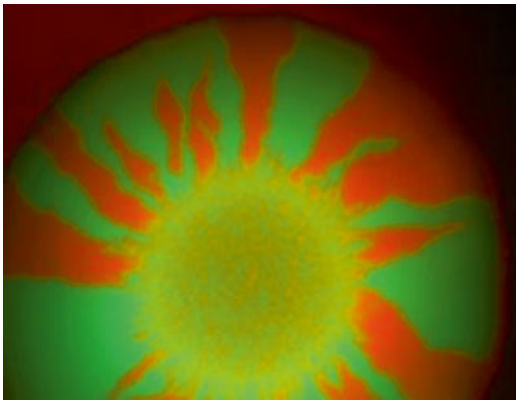

Fig. 2.15 Spatial distribution of evolving cell populations (From Hallatschek et al. 2007)

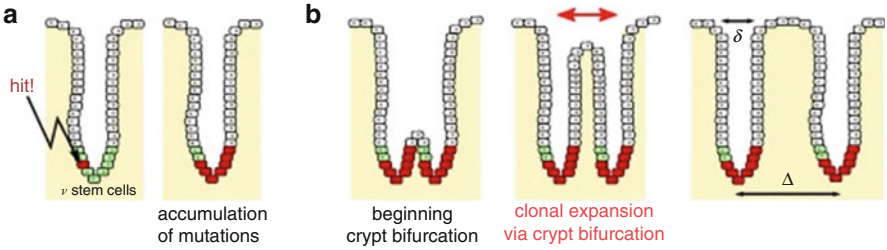

Fig. 2.16 Colon cancer model for understanding the growth of intestinal epithelial cells out of the crypt (From Martens et al. 2011)

bacteria. Built upon a salmonella backbone, they attempted to develop a bacterium that effectively targeted the tumor microenvironment, and could deliver a toxic payload. To accomplish this targeting they put an essential gene under the control of an oxygen sensitive promoter. Consequently, in presence of oxygen, the gene is not expressed and the bacteria die. Particularly notable, though exploiting evolution in their mechanism, the bacteria themselves were created by design and not by evolution.

We saw several notable examples of evolutionary studies in the work of Drs. Stefano Zapperi and Alberto d'Onofrio at the European Institute of Oncology, Italy (site report, Appendix B). Dr. Zapperi uses approaches very similar to those of Dr. Michor (branching birth-death processes). He has used these approaches to investigate the implications of cancer stem cells within a population (La Porta et al. 2012). Dr. Zapperi introduced a recent study on a novel approach to investigating tumor growth from a cancer-stem-cell perspective in melanoma. It is commonly believed that cell senescence—the loss of replicative capacity of cells—acts as a barrier for tumor growth. Dr. Zapperi and colleagues are investigating this phenomenon.

In their study, Dr. Zapperi and colleagues followed the evolution of senescence markers in melanoma cells and found that while most cancer cells eventually turn senescent, it is irrelevant for the long-term growth rate of a tumor. To demonstrate

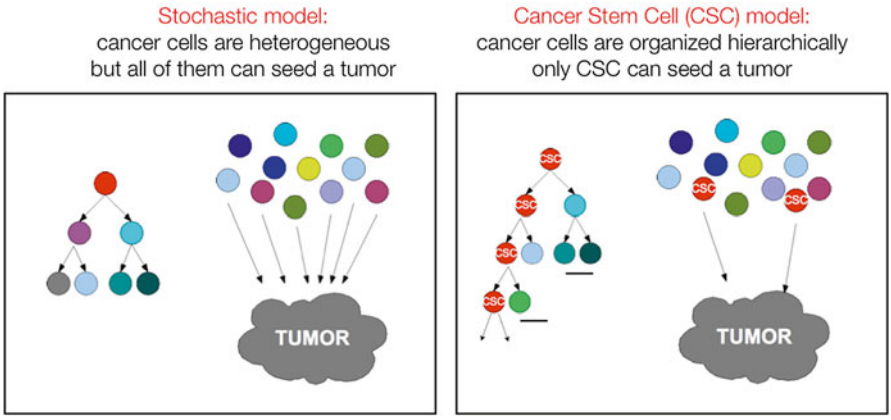

Fig. 2.17 Mathematical population dynamics model (From La Porta et al. 2012)

this phenomenon, they constructed a mathematical population dynamics model (Fig. 2.17, right) incorporating cancer stem cells, which is able to reproduce quantitatively the experimental data. Their results support the existence of cancer stem cells in melanoma and explain why it is difficult to fight cancer by inducing senescence in cancer cells. Only a fraction of the cells are susceptible to senescence, but those cells are irrelevant for tumor growth. A successful therapeutic strategy should instead target cancer stem cells, which are, however, likely to be strongly resistant to drug-induced senescence. This result is quite important and highlights the need of evolutionary modeling of tumor growth as well as the possible insights that come from formal modeling approaches. Notably, additional light on the existence of cancer stem cells has been provided by Drs. Clevers, Blanpain, and Parada (Schepers et al. 2012; Driessens et al. 2012; Chen et al. 2012).

Dr. d'Onofrio used similar approaches, but focused on the area of noise to develop strategies for optimizing anti-angiogenic therapies (Bertolini et al. 2011; d'Onofrio and Gandolfi 2010).

Discussion

We identified numerous examples of cancer behaving as a complex adaptive system throughout our study. Experimentally, this was observed at a variety of length scales from the single-protein to the tumor. Notably, this has engendered an impressive and diverse collection of modeling approaches. There is significant research being conducted abroad in all aspects of cancer as an information transfer system and of the evolutionary processes, state-evolution functions, and emergent properties. Among the major bottlenecks were a frequent need for close integration between experimentalists and modelers. In addition, to appropriately ask and answer a question about complex systems in biology, it was often necessary to design and perform

specific experiments and integrate those results with larger published data sets. We also observed a greater emphasis on the use of model systems (ranging from single proteins to yeast to cell culture) in Europe than we typically observe within the U.S. cancer research community. Unlike the United States where there is significant hesitation about non-clinically mimetic biosystems, that same hesitation did not appear to dominate European or Asian research. Notably, we also found significantly more emphasis on compact biomodel systems in Europe and Asia than in the United States. In Japan and Hong Kong, in particular, there was a significant emphasis on cellular-imaging approaches for getting incredibly high-resolution, views of cellular dynamical processes. There also was a significant emphasis on exploring specific types of effects and extracting principles that might be scaled up. This approach differed widely from the typical U.S. approach, which favors large-scale, global analyses. However, the advantage of global approaches is their ability to interrogate complex systems as a whole, rather than as a subset, such as in recent work to build total cell models (Karr et al. 2012). A major contributor to successful research endeavors was funding environment. Successful projects depended upon close collaboration amongst groups of researchers, particularly including a mix of experimentalists, bioinformaticians, and modelers. Consequently, multi-investigator funding mechanisms have been critical for pushing innovation at the frontier of information transfer and complex systems analysis of biology. Through programs, such as those engendered by the NCI's Office of Physical Sciences Oncology, and foundation awards at the interface between the physical and life sciences, investigators in the United States have been fortunate to have access to interdisciplinary funding opportunities. Generally, we conclude that the areas of information transfer, evolution, and complex adaptive systems research are rapidly progressing, and critically important for impacting cancer and more generally understanding biology.

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