Introduction

It has become clear that developmental processes that guide tissue formation and failures in that process that can lead to tumorogenesis are driven by integrated modalities ranging from relatively simple chemical kinetic reactions to adhesion-mediated cellular shape changes and mechanically-induced nuclear reorganization. New and improving single cell imaging techniques such as optical sectioning microscopy (confocal and convolution) and soft x-ray microscopy allow unprecedented querying of cell shape and cytoskeletal organization and tracking of protein expression and localization. Cell and molecular biological techniques have advanced to the point that two and three dimensional cultures of mammalian tissue can be finely manipulated genetically and immuno-chemically in order to investigate the role of ECM, cell-cell adhesion and various signaling pathways on the ability of individual cells to properly form healthy tissue and control their own shapes and growth. However, there has not been a comparable increase in the quantitative understanding of how mechanical, cytochemical, and genetic processes interact in order to produce the orderly three dimensional growth of tissue and how disruptions of the various components can lead to be reversible and irreversible failure of this process.

Just as the advent of the mathematics for describing enzyme kinetics has immeasurably aided not only the quantitative but the qualitative understanding the control and functioning of metabolism, theoretical models for how the cell senses its mechanical and chemical environment as well as its own shape will aid in understanding the immeasurably more complex field of morphogenesis. Though a theoretical description of the entire process from cell-cell contacts to nuclear-reorganization and gene transcription is not yet possible there are a number of questions which can now, given the technological advances cited above, be addressed theoretically in order to disentangle the complex web of cause and effect in these processes:

- Can mechanical forces and shape changes be transmitted to the nucleus directly through coupling to elements in the cytoskeleton that impinge upon the nuclear matrix?
- If so can such forces cause significant changes in chromatin structure such that gene transcription patterns are effected?
- In what ways, other than through stretch-sensitive receptors, can mechanical forces be transduced into chemical activity?
- How much change is there in the rheological, percolation, and network structural properties of the cytoskeleton upon externally applied force and how do we distinguish force induced changes from biochemically induced changes?
- In what ways can changes in the cytoskeletal and nuclear geometry effect chemical kinetics?
- Can cellular shape changes be explained wholly through cell-cell adhesion forces and if not, how does one distinguish between activation of internal shape-change processes and purely cell-mechanical effects?

Below we describe various problems, approaches and prior art in the theoretical literature that may aid in answering these questions.

Problems and Approaches

Nearly all the approaches discuss herein rely on the ability to form physically reasonable models of receptor-receptor, receptor-cytoskeleton and cytoskeleton-cytoskeleton interactions. Such models are becoming possible both due to the high-resolution imaging technologies cited above and the increasing number of measurements on the structural, mechanical and chemical properties of actin gels and fibers, single and groups of microtubules and intermediate filaments and the rheological properties of cells.

Aggregation induced shape change

These studies concern whole cell including cell motility, cell adhesion, cell-cell, cell-ECM interactions that lead to physical shape changes and may lead to force production or tension on the cytoskeletal networks inside the cells. Because adhesion and force generation is intimately tied to receptor localization, the effect is included in this section.

Adhesion mediated receptor localization.

It is known that ligands of integrins can cause both integrin aggregation as well as co-aggregation with growth-factor receptors. Upon aggregation of these receptors there is a synergistic activation of tyrosine-phosphorylation pathways that can carry the information about these adhesion events to the nucleus and modulate gene expression. The physics and kinetics of receptor aggregation in lipid bilayers is amenable to theoretical investigation. The role of the distribution of ligands in the cellular supporting stratum, the ECM and on neighboring cells provides geometric and kinetic constraints on the way in which these receptors aggregate and thus can transmit the signal. In addition, it is known that receptor-cytoskeletal interactions affect the formation of these receptor clusters which also serve as precursors to mature focal contacts. The ability of cytoskeletal elements to recognize one another above a critical concentration may be a driving force for this aggregation process. Externally mediated cellular shape changes may change the local density of cytoskeletal elements and thus directly effect the formation of the receptor aggregates.

Cell Motility and Traction Models

Experimentally it is possible to measure the force and traction pattern of locomoting cells on a defined substrate. When cells are forming tissues they move by traction both on the substratum and to each other. The pattern of attachment and force exertion defines the sorts shapes that cells can adopt when in contact with other cells that are also moving, adhering and pulling. Cell mechanical models can be used to determine the spatial distribution of forces necessary to achieve proper cell shape and attachment and to explore quantitative the effect of disrupting adhesion and traction forces through, for example, the application of antibody ligands for the integrins.

Shape-Change induced Actin/Tubulin Reorganization

When the outside shape of the cell changes due to external forces or traction of the cell on outside surfaces, the local structure of the cytoskeletal networks must change as well. Changes in this network could have a number of effects: the mechanical properties of the cell might qualitatively change (e.g. by cooperative interactions between cytoskeletal elements); Membrane-associated and cytoskeletally linked proteins might change their aggregation state leading to signal transduction; network bound cytosolic proteins might change their local densities in order to speed or decrease the rates of certain reaction; ‘free’ cytosolic chemically might change their diffusion properties or other transport rates; and cellular compartments might be reshaped and stressed. This section describes a few approaches to these problems.

Adhesion induced signal transduction

This is the cytosolic component of the adhesion mediated aggregation section described above. Signal transduction by adhesion can occur via a number of modes: channels can be forced to open (or close), adhesion may change the conformational or dynamical receptor structure that may be transmitted mechanically to the cytoplasmic side of the protein in order to induce chemical reaction,. or adhesion might exert local force on the cell inducing reorganization of the local cytoskeletal network which itself might be responsible for changing enzymatic reaction rates. Simplified models of protein aggregation in membranes under stress and models with an without receptor/cytoskeletal interaction may lead a novel picture of adhesion induced signal transduction.

Network reorganization induced reaction rates

This study differs from the one above in that it focuses on the cytoskeletal network as a reaction medium. This medium is unique in that it is highly structured, may have very different binding affinities for the ‘solutes’ and may lead to precise geometric arrangements of reaction intermediates. Changes in porosity, density, branching and global geometries due to mechanical and chemical remodeling processes may induce

very non-classical kinetics for the enzymes systems associated with the matrix. Simple models of cytoskeletal network structures (based on the microscopy evidence cited above) can be used as a scaffolding onto which may be applied arrangements of kinases and kinase targets, diffusable messengers, etc. Whose kinetics may be modeled using standard finite-element techniques. Results from the models can be directly compared to kinetics measured in *in vitro* cytoskeletal extracts and in some cases to direct single cell measurements.

Network Reorganization nucleus/chromatin restructuring

One open question is whether adhesion and traction forces generated by the cytoskeleton induce changes in nuclear conformation that may be ordered enough to induce or repress particular gene expression systems.

Nucleus/Chromatin restructuring induced gene expression

The first step in showing that mechanical forces can be directly transduced to the nucleus is the use models developed above of the cytoskeleton and the mechanical properties of its elements to construct a broad-brush mechanical model of a whole cell in which changes in cell shape and forces on the outside of the cell can be transmitted through the cytoskeletal matrix to impinge upon a model of a 'structured nucleus'. The model of the structure nucleus can be partially derived by halo experiments and detail optical sectioning microscope images of the arrangement of nuclear matrix associated proteins, histone distribution and chromatin geometry. Rough estimation of the force on the structures that is transmitted to the nucleus may be achieved through finite-element modeling. Estimation of changes in internal structure of the nucleus due to these forces will require estimates of how tightly DNA is coiled around the histones and wrapped into chromatin, and then how tightly these are attached to the nuclear matrix. Part of this information can be garnered from studies like suggested in the next section.

Chromatin/DNA mechanics and dynamics/ effects of acetylation

The precise forces which wind DNA around histones are not fully known. However, the three-dimensional structures of histones and nuclear-matrix protein/DNA complex have recently been determined and so physical models can be made for the dynamics of the interaction. In addition, informatic efforts have identified sequence motifs that attach to the nuclear scaffolding and dynamical models have been produced that attempt to construct a dynamical model of assembly of heterochromatic regions in position effect variegation. Thus mechanical models of DNA/protein association can be made to: estimate the amount of force necessary to remodel chromatin enough to allow DNA transcription; estimate the effect on DNA binding and the aforementioned forces of modifying nuclear DNA binding protein by phosphorylation or acetylation; construct qualitative models of nuclear reorganization upon application of extracellular forces. Such models provide physical standards to which hypotheses about mechanical and spatial inputs to developmental processes must be compared.

Summary

There is an opportunity to apply mathematical analyses to all levels of adhesion and force-mediated shape changes and developmental switching in potentially tumorogenic cells. These range from somewhat abstracted models of cell motility and traction generation, to more detailed models of cytoskeletal network organization and chemical kinetics, to molecularly detailed models of DNA/histone interactions. These analyses use and explain data being generated from the advanced cell and molecular biology facilities, the advanced microscopy facility and the bioinformatics core at the National labs and thus represents a central processing points for the diverse information being generated at these disparate sources. However, the pay-off of quantitative examination of this data, data that indicated a strong interaction between mechanical cellular environment and developmental and oncogenic processes, is large. Many incremental problems can be approached immediately and are necessary precursors for solving the larger problem of tissue to gene regulation of development and disease.

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