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Determining Physical Principles of Subcellular Organization

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Recent advances have transformed our understanding of cell biology, but we are still unable to predict the behaviors of these systems. One difficulty is that we lack an understanding of the physical principles of subcellular organization. Combining quantitative experiments with new theoretical insights may allow such principles to be developed.

There have been astonishing advances in cell biology over the last sixty years. For young scientists such as ourselves, who came to biology within the last decade, it is shocking to realize that the foundational discoveries that have clarified the basic workings of cells and led to the identification of the major molecular constituents of many cellular processes are so recent. In our own field of cell division and the cytoskeleton, it was only in the 1970s that tubulin was shown to form microtubules, while the microtubule molecular motors dynein and kinesin were identified in the 1960s and 1980s, respectively, and many of the other major molecular constituents of the spindle were discovered even more recently (Neumann et al., 2010). As late as 1944, Schrader, in his classic review of mitosis, discussed models that modern readers would view as absurd: that the spindle might not be made of filaments, but rather hydrodynamic flows organized by pulsating spheres, ions arranged by electric fields, or other possibilities. While Schrader was quick to reject these ideas—"The viewpoint is that of a physicist, without any reference to biological phenomena" (Schrader, 1944)—it is telling that he still felt it necessary to mention them. The pace of advance continues to accelerate: with the advent of cheap genome sequencing and high-throughput genetic manipulation, it is becoming feasible to systematically determine the molecular components that contribute to a wide range of cellular processes. In the last decade, there have been multiple genome-wide perturbation studies of cell division in *C. elegans*, yeast, *Drosophila*, and human tissue culture cells, increasingly leading to the sense that nearly all the proteins that contribute to mitosis have been discovered (Neumann et al., 2010).

However, despite this remarkable progress, a fundamental understanding of even the most well-studied cell biological processes is lacking, as evidenced by our inability to make predictions of their behaviors. The clearest, and most immediately consequential, demonstration of this lack of predictive power is the current difficulties of the pharmaceutical industry. In the past 60 years, the amount of money spent on drug research and development has skyrocketed while the success rates of these efforts have plummeted, causing widespread concern for the viability of the industry (Scannell et al., 2012). The reasons for this decline are not agreed upon, but some observers contend that it is due to the modern emphasis on hypothesis-driven, targeted approaches based on molecular reductionism (Swinney and Anthony, 2011). Even if attempts to apply the current understanding of biology are not responsible for the crisis in drug development (after all, there have been a number of clear successes of this approach, such as the advent of drugs for HIV), it is still disappointing that these efforts have not resulted in more progress in the development of new drug therapeutics. This situation is in contrast to areas of physics, such as material science, mechanical engineering, and solid-state physics, where the understanding from basic science has consistently been used to produce remarkable improvements in performance and drastic reduction in price for a range of applications. Predictive theories of cell biology would not only aid in drug development and other medical applications (such as diagnostics and prognostics), but would also greatly empower synthetic biology and provide a basis for mechanistically understanding evolutionary change. More fundamentally, the ability to make predictions would be the most convincing evidence for the validity of proposed explanations for cell biological phenomena.

Why are we still unable to make predictions in cell biology despite all of the progress that has been made? It is sometimes said that the difficulty is that, while we know the proteins that contribute to different process, we do not know how these proteins interact. There is certainly truth to this, but it obscures the magnitude of the problem. The issue is not simply that we need to characterize pairwise (or three-body, or four-body) binding interactions between proteins, but rather that cells consist of extremely complex, spatially heterogeneous, partially ordered, dynamic assemblies. Such structures, based on the cytoskeleton, membranous systems, and non-membrane-bound protein "bodies" (Figure 1), underlie much of metabolism, secretion, signaling, motility, division, and gene expression, but the behaviors of these systems, which are large compared to proteins, yet small compared to cells, remain poorly understood. Thus, one of the major challenges is that we lack

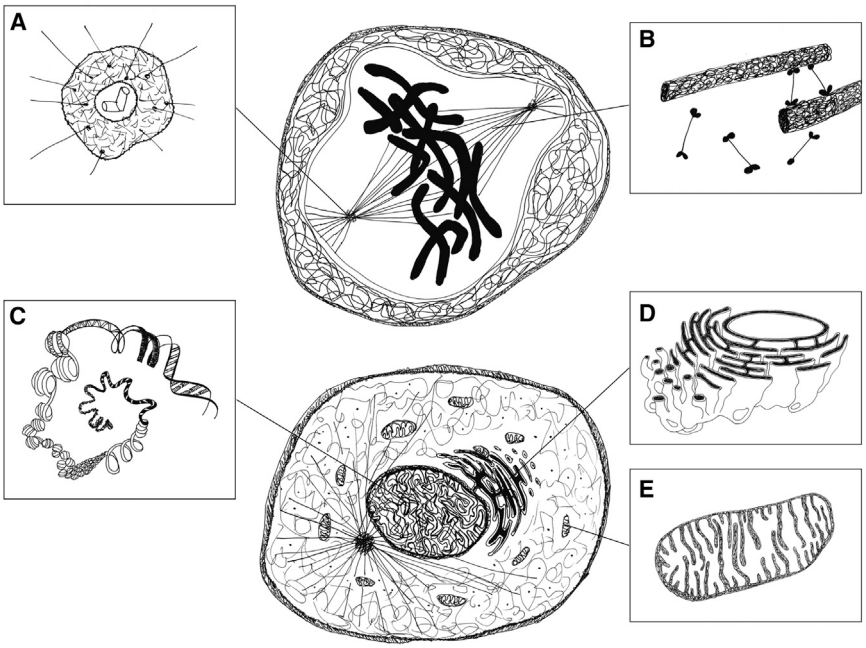

Figure 1. Subcellular Organization
(A–E) Complex structures underlie subcellular organization in metaphase (upper) and interphase (lower). (A) The centrosome is a non-membrane-bound protein assembly. (B) Microtubules, motors, and other proteins interact to assemble the spindle, which segregates chromosomes during cell division. (C) Chromatin organization influences gene expression. (D) The structure and dynamics of the Golgi apparatus play major roles in protein secretion. (E) Mitochondrial metabolic activity is determined by an interplay between membrane-bound, compartmentalized, and soluble factors. Figure drawn by Julia Eichhorn.

predictive theories of subcellular organization above the molecular level.

During the first half of the 20th century there was a substantial amount of work on cell organization that, by necessity, focused on scales above the molecular level and sought to explain biological behaviors based on the material and mechanical properties of cells (beautifully reviewed by Pickens, 1960). This program was largely abandoned by mainstream biologists as it became unclear how to make progress when so little was known about the constituents of cells and with the realization that their physical properties were quite different from substances that were well understood, such as simple solids and liquids. Since then, the components that make up many subcellular structures have now been established, whereas the development of soft condensed matter physics has led to a sophisticated understanding of polymers, liquid crystals, membranes, and other biological materials. While soft condensed matter physics has produced numerous deep insights and provided the basis for a range of industrial applications, these advances in material physics have, so far, not had a broad impact on the understanding of subcellular organization. Exciting developments indicate that this trend may change, as researchers have begun to incorporate detailed biological knowledge with established principles from soft condensed matter physics to attempt to gain insight into a range of subcellular structures, such as using polymer physics for understanding nuclear organization (Fudenberg and Mirny, 2012), the physics of phase transitions for non-membrane-bound macromolecular assemblies (Brangwynne, 2013), and membrane mechanics for organelle shape (Shibata et al., 2009). This work is particularly promising because much of it is closely tied to the interpretation of new experimental data.

However, there is a fundamental difference between materials traditionally studied in soft condensed matter physics and subcellular structures: unlike their synthetic analogs, many of the biological molecules that make up subcellular structures consume chemical energy to produce conformational changes, power chemical reactions, and perform mechanical work. Collections of such "active," energy-consuming molecules can exhibit behaviors that are impossible for collections of their "passive" counterparts. Steady-state structures that spontaneously form from active molecules are said to be "self-organizing," in contrast to spontaneously forming structures composed of passive particles, which are said to "self-assemble." The self-organization of "active matter"—collections of active particles—is different from other nonequilibrium steady-state structures that have been more extensively analyzed, such as those that arise from hydrodynamic instabilities—where a conventional material is forced out of equilibrium by a macroscopic, external drive, such as a fluid heated from below, producing Benard rolls (regular patterns of the rising and sinking of the fluid) —or spatial inhomogeneous "dissipative structures," which can form in systems of chemical reactions. In contrast, in active materials, energy flows in through the microscopic degrees of freedom, at the molecular level, and involves mechanical as well as chemical activities. Experimental and theoretical studies of the behaviors of active matter are quite recent, and it is still unclear to what extent concepts developed in this field can be profitably applied toward understanding cell biology. Thus, researchers are presently tasked with the dual challenges of simultaneously discovering general principles of the behaviors of active matter and establishing if these principles can be used to explain specific cell biological phenomena.

There is a long history of interplay between physics and biology in which biological phenomena inspire the development of new areas of physics, which are in turn used to understand biology. In the 19th century, the physician Jean Poiseuille performed detailed experiments on the flow of liquids in thin pipes in the hopes of providing a foundation for understanding blood flow. This work helped establish the validity of the Navier-Stokes equations, the theory of fluid motion that is now widely used in a range of applications, including in physiology. Studies of heat generation by animals separately led both Robert Mayer and Hermann von Helmholtz to propose the principles of conservation of energy,

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which, as part of the basis of thermodynamics, is crucial for our understanding of metabolism and, more broadly, of all biochemistry. The idea that ''animal electricity'' might be the vital force that produces life motivated early studies of electricity, a line of inquiry that ultimately led to the development of the first batteries (called ''artificial electric eels'') and the discovery of electrical currents. This work came full circle in the 1950s with the research of Hodgkin and Huxley, in which they used quantitative experiments and electric circuit theory to produce a sophisticated, mathematical understanding of the propagation of electrical activity in neurons.

What about cell biology? Will it be possible to develop physical principles of subcellular organization to help establish predictive theories of cell biology? If so, will theories of active matter contribute to this process? It is still too early to tell, but the realization that many cellular structures, such as the spindle, the nucleus, centrioles, the Golgi apparatus, and even cell morphology, are likely self-organized (Karsenti, 2008) inspires confidence that it might be possible to develop general principles to elucidate what these disparate systems fundamentally share in common. Only once predictive theories of particular systems are established will it be possible to compare different systems to develop generalities.

Currently, the most extensive efforts on understanding self-organization and active matter have focused on collections of cytoskeletal filaments, which underlie processes such as cell motility and cell division. Three lines of research have addressed these issues:

(1) Studies of complex assemblies in cells and cell extracts (Karsenti, 2008). Such work discovered the behaviors of cellular systems that need to be explained, established the idea of self-organization in cell biology, and identified many of the molecular constituents of these processes.
(2) In vitro reconstitutions of purified components (Nédélec et al., 1997; Sanchez et al., 2012). Experiments on mixtures of cytoskeletal filaments, molecular motors, and other components have demonstrated that these highly simplified systems are capable of self-organizing into patterns reminiscent of cell biological structures.
(3) Theories of the behaviors of ensembles of cytoskeletal filaments. There are two main theoretical approaches to describe self-organization of cytoskeletal systems: microscopic descriptions based on explicit interactions between filaments and motors and generic description based on coarse-grained variables, such as mass and momentum densities and filament orientation (Marchetti et al., 2013). Both descriptions aim to describe the active nature of the cytoskeleton, which results from the continuous consumption of energy by the polymerization dynamics of filament and motor activities.

The starting point of the microscopic descriptions is the set of rules by which motors slide on filaments, creating motion. These descriptions can also include the polymerization dynamics of the filaments. Considering the averaged effects of multiple such microscopic interactions results in a theoretical description in terms of phenomenological parameters that can be traced back to microscopic parameters such as motor activity or filament concentration. The strength of these theories is that they allow for a direct connection between the large-scale behaviors of the cytoskeleton and its molecular constituents. The main limitation of these theories is that we currently know very little about the actual microscopic behaviors of the molecular constituents or rules of interactions between cytoskeleton filaments. Therefore, it is difficult to construct realistic microscopic theories, and it is challenging to know the extent that their predictions depend on potentially faulty assumptions.

The goal of generic descriptions based on coarse-grained variables is to capture the long timescales and large length scales of the cytoskeleton and are valid for length scales larger than the size of its microscopic constituents (filament length). These theories aim to describe the behaviors of the system around a steady state, and the resulting theories are analogous to formulating Navier-Stokes-like equations for the cytoskeleton. To derive the terms contained in these theories, a linear expansion containing all possible terms in gradients allowed by symmetry is included, with strengths encoded by phenomenological parameters. Although these theories are general, in that they include all possible terms consistent with the coarse-grained variables of the system, their main difficulty is the resulting large number of phenomenological parameters, which may limit their predictive power. For systems near equilibrium, these phenomenological parameters are reduced due to thermodynamic considerations. The finite size of the systems may also limit the applicability of such gradient expansions, as well as the possible necessity to incorporate nonlinear terms, such as the manner in which motor activity or polymerization is modified by forces, which are based on effects outside the linear regime. The phenomenological parameters have no direct connection with microscopic details, so each parameter has to be measured experimentally. Thus the main challenge of both microscopic and hydrodynamic descriptions resides in designing quantitative measurements to test and validate these theories.

While great insight has been obtained from studying complex cellular structures, simplified in vitro systems, and developing theories of collections of cytoskeletal filaments, these three approaches have still not been fully integrated, but such efforts are currently being pursued by many research groups. In vitro reconstituted systems can be used to demonstrate that select purified components are sufficient to recapitulate aspects of cell biological structures, and, if their microscopic interactions can be well characterized, these systems can also be used to rigorously test theories of how these interactions produce collective behaviors. Quantitative measurements and experiments on cell biological structures will further allow direct tests of the validity of theories of these systems. The marriage of these three approaches holds the promise of establishing physical principles of subcellular organization and producing truly predictive theories of biology.

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