David K. Lubensky: Research Statement

May 28, 2018

I do theoretical and computational research at the interface between physics and biology, with a focus on the collective behavior of groups of interacting genes, proteins, and cells. Over the past half century or more, biologists have become remarkably adept at dissecting the properties of individual genes and proteins. These basic biological units, however, do not exist in isolation; a typical human cell has tens of thousands of genes and many times that number of different kinds of molecules, all bumping up against one another in a region smaller than the head of a pin. On its face, this might seem like a recipe for disaster: Were one to mix an assortment of thousands of randomly chosen chemicals in a beaker, the best result one could hope for would be a nondescript brown goo (or perhaps an explosion). Yet, miraculously, when a comparable variety of biological molecules are brought together inside a cell, they instead arrange into the myriad different systems that together sustain life (Fig. 1). Moreover, they manage to configure themselves in this way without any centralized control—there is no master builder to reach in and place each protein at its appointed location in a cell or to sculpt a group of cells into a human kidney or hand. Instead, by virtue of their interactions with each other, these individual constituents are able to *self-organize* into the correct patterns in space and time. I want to understand the physical principles governing this process, at both the subcellular and supracellular scales. **The fundamental question I seek to answer is what special properties of living matter distinguish it from non-living systems and allow it to attain the exquisite degree of self-organization, functionality, and robustness that we observe in nature.**

Although this might seem like a strange topic for a theoretical physicist, in fact self-organization is a subject with a long history in the physical sciences. The ways that atoms condense into regular, ordered crystals or that convection rolls in fluids form spatio-temporal patterns have been studied for well over a century. In my work, I apply the theoretical tools developed to describe these physical systems to the still more intricate forms of organization observed in biology.

In practice, this means that I build mathematical models of specific biological systems, chosen for their potential to serve as paradigms for a wider set of phenomena. I then try to distill more broadly applicable ideas and principles from what I have learned about these systems, and when possible to give these ideas a precise mathematical expression. I ground my models firmly in the available experimental data and strive to make experimentally testable predictions. In fact, when possible, I prefer to collaborate closely with experimental biologists, and my research group puts considerable effort into developing image processing and statistical tools to analyze the data produced by these collaborations.

Fig. 1: One likely result of mixing thousands of different randomly chosen chemicals (top), and the outcome when a similar variety of substances come together in a living cell (bottom). I study what special features of the interactions among biological molecules make possible the remarkable degree of organization and functionality seen in the cell.

In recent work, I have studied examples of animal development[1-5]—the transformation of a single-celled egg into an adult animal—and of circadian clocks[6-13]—the internal timekeepers that regulate everything from when we wake up to which times of day we run the fastest or concentrate the best. Among my major contributions from the past 5 years are:

- The refinement and testing of my earlier model of cell organization in the zebrafish retina. Most notably, we were able to directly demonstrate the presence of anisotropic mechanical stresses that the model predicts are central to pattern formation during this system's development[1,4].
- The discovery that topological features—the tricellular junctions—in epithelial sheets of cells can be used to "memorize" a cell's geometry, allowing it to correctly orient its division axis[2].

- A demonstration that the same pathway that picks out a preferred direction in cells can also drive spontaneous left-right symmetry breaking, potentially explaining the appearance of handedness in many systems[5].
- A proof that cellular growth and division should generically disturb circadian clocks and an explanation of how the bacterium *S. elongatus* is able to mitigate this effect[6].
- Studies of the interplay of noise and entrainment in determining biological clock performance, including arguments establishing that true, autonomous clocks always outperform other timekeeping systems when environmental fluctuations are the dominant source of noise[10,11].

Animal Development

Development is the process by which a fertilized embryo grows into an adult organism. Developmental biologists thus study how an egg knows what it wants to be when it grows up—and how, given this knowledge, it is able to reliably transform itself into an incredibly complex adult animal. To a physicist, this transformation is a stunning example of self-organization. I want to know how the animal's final form is encoded in the interactions among its cells. **In particular, I am interested in how mechanical forces and biochemical signals conspire to determine the shape and spatial arrangement of cells in epithelia, the sheet-like tissues of tightly conjoined, roughly polygonal cells that make up everything from the skin to the intestinal lining**. In recent years, I have worked with experimental groups to examine cell organization in the epithelia of the zebrafish retina[1,4] and the fruitfly dorsal notum[2] and have studied models of the stability of junctions where four different cells come together[3] and of left-right symmetry breaking[5].

Cone cell packing in the zebrafish retina

The zebrafish retina contains a strikingly regular, crystalline arrangement of cone photoreceptor cells (Fig. 2) that emerges during larval development. My experimental collaborator Pamela Raymond and I have used this system as a paradigm for the conversion of a disordered cell packing into a highly ordered pattern. In an earlier paper, we proposed a model for this process that relies on a coupling between anisotropy in tissue-scale mechanical stresses and biochemically-driven rotational symmetry breaking within individual cells. To extend this work, we used the *tbx2b* mutant to gain a deeper understanding of the exact sequence of steps that lead to the formation of the regular cone mosaic[1]; in particular, we demonstrated that cell-cell interactions remain strongly directional, even when global order is lost, in accordance with our model, and we predicted and observed that the *tbx2b* phenotype can be explained by the loss of UV-sensitive cones at a key stage of development. Subsequently[4], we employed laser ablation to directly confirm our central hypothesis of strongly anisotropic stresses in the retinal epithelium. Moreover, we showed that, unexpectedly, the main source of this anisotropy is not the cone cells themselves, but laminar Müller glial processes that encircle each photoreceptor. In both of these papers, my group made major contributions not only to model development but also to the quantitative analysis of image data. These included finding creative ways to measure the anisotropy of cell clusters in mutant epithelia and to extract clear evidence of oriented ridges of glial processes far earlier in development than previously thought. Taken together, our work on the zebrafish retina has allowed us to understand, in considerable detail, a prime example of the emergence of ordered cell packings.

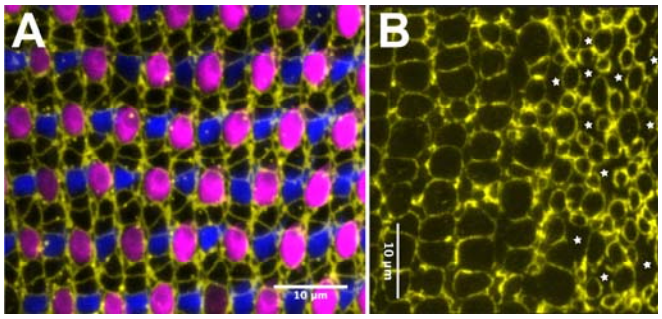

Fig. 2: A) The crystalline mosaic of cone photoreceptor cells in zebrafish retina. Cells are outlined in yellow. Blue-sensitive cones and those sensitive to UV light (magenta) are labelled. Unlabeled cone cells are sensitive to green or red light. B) The emergence of the cone mosaic at the margin of the retina. A disordered cell packing (right side) abruptly develops into regular columns of cells (left side). (Stars indicate cells that are not cone photoreceptors.)

Orienting cell divisions in epithelia

Mechanical anisotropies influence not only the rearrangements of epithelial cells, but also their division: it has long been known that cells elongate in response to applied forces and then tend to divide perpendicular to their long axis. Paradoxically, however, these same cells adopt a rounded configuration immediately before division, so that their shape at the moment of splitting provides no information about the correct division orientation. It thus is a long-standing mystery how cells reliably know in which direction they should divide. Working with the lab of Yohanns Bellaïche, I showed that, in the developing dorsal notum of the fly pupa, the key is the use of tricellular junctions (TCJs) where three cells meet (Fig. 3)[2]. Even as the shape of a given cell changes dramatically with the approach of division, the multi-cellular packing constrains the movements of the TCJs so that their distribution around the dividing cell's boundary still reflects the initial long axis direction. Thus, strikingly, objects with a topological flavor—the TCJs—are used to memorize a purely geometric feature—the average axis orientation. The direction of cell division guides organs' growth to their correct adult form in animals from worms to humans, so understanding how this orientation is determined has implications for many fundamental problems in biology and medicine.

Stability in vertex models

One widely-used class of cell-level descriptions for epithelia are the "vertex models," which treat cells in the epithelium as polygons whose vertices move in response to applied forces. Given their popularity, there has been remarkably little study of their basic mathematical properties. Typically, planar polygons meet at 3-fold vertices (the TCJs described above), but apparently stable vertices joining four or more cells are sometimes seen in real epithelia. It is thus natural to ask when stable fourfold or higher order vertices are possible in a vertex model. We derived general vertex stability criteria and found that fourfold vertices are never stable in the simplest models, in which all polygon edges have the same mechanical tension, but that stability becomes possible with biologically-plausible extensions like orientation-dependent tensions. This work also established a rigorous basis for the numerical treatment of topological changes that require transient fourfold vertices[3].

Left-right symmetry breaking

Essentially all animal body plans have some degree of left-right asymmetry. For example, the human heart tilts towards the left while the appendix sits in the lower right corner of the abdomen. As an embryo grows into an adult animal, it must put these organs on the correct side of the body, and it is not well-understood how it does this. Drawing on an analogy with the physics of liquid crystals, we demonstrated a new mechanism by which developing organs can acquire a specific handedness by coopting proteins that naturally congregate only on certain edges of a polygonal cell (like those in the well-known planar cell polarity pathway)[5]. Such systems will typically go through a chiral phase as the fraction of total protein that can fit on a given edge varies. This mechanism can explain a number of puzzling observations without the need to invoke hypothetical, uncharacterized, genes or proteins.

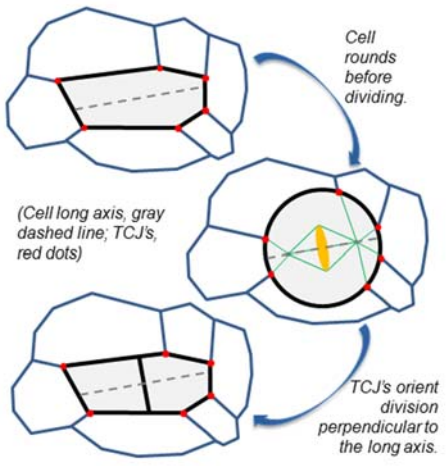

Fig. 3: Mitosis in epithelia. Before dividing, a cell rounds up (middle), so that its current shape provides no information as to the orientation of its long axis before rounding. This orientation is still reflected, however, in the locations of the tricellular junctions (TCJs, red dots), allowing the cell to orient its division plane correctly (adapted from Ref. 2).

Circadian clocks

Living matter displays exquisite organization not only in space but also in time. My second major research focus[6-13] addresses this temporal order, exemplified by the circadian clocks that allow organisms to

anticipate daily light-dark cycles. (That these clocks resist sudden resets accounts, for example, for the existence of jet lag.) Circadian clocks are autonomous oscillators, built from networks of interacting genes and proteins, that entrain to daily variations in light or temperature but that can continue to count out their 24 hour cycles even in perfectly constant conditions. Remarkably, the circadian clock of the photosynthetic bacterium *S. elongatus* can be reconstituted in a test tube using only purified proteins. This poses the compelling question of how to build an oscillator solely from interactions among the three proteins KaiA, KaiB, and KaiC. In an initial study, my collaborators and I showed that intrinsically cyclic enzymatic reactions, of the sort sustained by KaiC, are natural building blocks for a novel class of "molecular synchronization" oscillators. **Over the past 5 years, we have built on this work by deepening our understanding of the molecular mechanisms behind the Kai oscillator and by using the Kai system as a starting point to examine how noise and perturbations constrain the design of biochemical oscillators.**

Dissecting the post-translational clock in S. elongatus

The *in vitro* Kai clock keeps time by cyclically attaching phosphate "tags" to KaiC and then removing them, with KaiA and KaiB modulating the rates of these reactions to synchronize the phosphorylation states of different KaiC molecules. Like any chemical oscillator, the Kai system depends on a source of energy, in this case ATP, to maintain itself out of equilibrium. How exactly ATP consumption maintains oscillations, however, has turned out to be surprisingly complex. Very unexpectedly, it turns out that the KaiC dephosphorylation reaction is the exact reverse of the phosphorylation reaction and so does not consume ATP. The phosphorylation cycle thus cannot by itself sustain non-equilibrium fluxes, necessitating a complete rethinking of how ATP drives the clock. To this end, we built a detailed, biochemically faithful and thermodynamically consistent model of the Kai system. By comparing its behavior with a wealth of experimental data, we concluded that the essential thermodynamic driving force is the use of ATP hydrolysis to induce conformational changes in KaiC[7]. As the most accurate and complete description of the Kai system available, our new model is also a powerful tool to investigate a variety of other questions. So far, we have used it to predict that the Kai-based clock is capable of a novel form of temperature compensation in which temperature-dependent shifts in reaction rates are balanced by changes in the length of the cycle each protein traverses in state space[8]. It also served as one of several examples in a study that explored how to employ models to constrain reaction rates that cannot be measured directly. We showed how the addition of proteins that compete with the Kai proteins for binding sites can be used to infer the strength of a key feedback loop in a manner that is independent of many biochemical assumptions. We could thus convincingly demonstrate that the Kai oscillator is a so-called "delay oscillator" that depends only on negative feedback rather than a "relaxation oscillator" that requires a balance of positive and negative feedbacks[13].

Coupling between clocks and the cell cycle

Ultimately, the Kai clock must carry out its tasks not in isolation in a test tube, but inside a living cell. One is thus naturally lead to ask how the myriad other processes occurring in this environment affect the clock. We showed[6,9] that cell growth and division must generically disturb biological clocks, especially in bacteria: As each clock gene in the genome is replicated, its number of copies per cell doubles almost instantaneously (Fig. 4). If the cell division time is comparable to the clock period, this amounts to a periodic forcing of the clock circuit. As oscillators are known to be especially sensitive to such driving, the clock should show a much stronger response to this perturbation than other genetic circuits (Fig. 4D). The nature of

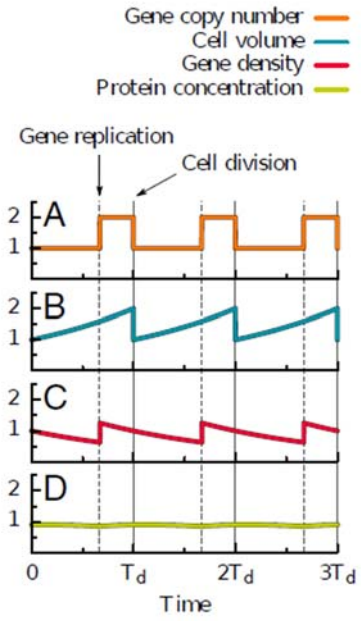

Fig. 4: Copy number of a gene (A), cell volume (B), and gene density (C) versus time for a cell growing with doubling time T_d. The gene is replicated once per cycle, causing a step increase in concentration. This has little effect on the concentration of a constitutively expressed protein (D), but strongly affects a clock[6].

this response, however, turns out to depend in subtle ways on the clock architecture and on superficially unrelated features of the cellular environment. For example, the fact that *S. elongatus* contains several identical, asynchronously replicated copies of its chromosome effectively insulates its circadian clock from many of the effects of the cell division cycle[6]; this conclusion might help to explain why evolution has selected for this unusual feature. The same sort of cell cycle driving can also affect synthetic oscillators introduced into bacterial cells through genetic engineering. Indeed, we found that oscillators built from the same components can be disrupted in very different ways depending on the order in which the genes are integrated into the chromosome[9]. Together, these findings shed light on the larger issue of "embedding": When can a functional subcircuit within a cell be considered (to some approximation) in isolation, and when must its coupling to other systems be taken into account?

Optimal design and the adaptive value of clocks

That all biological clocks are necessarily imperfect, as our work on embedding emphasizes, hints at a still broader question: Why have a circadian clock at all—when is the advantage it confers worth the resources it takes to create and maintain it? Even without any way to tell the time, an organism can still respond to changing light levels as it detects them, so clocks are usually presumed to provide an advantage by allowing cells to anticipate future changes in their environment. To understand how helpful such anticipation could be, we adapted coarse-grained models of bacterial metabolism and growth to consider the specific case of nitrogen-fixing cyanobacteria, where we concluded that the ability to prepare for the transition from day to night can lead to increases of 20% in the average bacterial growth rate[12]. Thus, in this case, there is a clear reason to have some time-keeping system. Even then, however, it is not obvious that organisms necessarily need a true clock that will continue ticking indefinitely even without daily environmental input. Indeed, while some photosynthetic bacteria have real circadian clocks, others appear instead to have "hourglasses"—that is, systems that can keep track of elapsed time for 12 or 24 hours but then must be reset by some stimulus (like a change in the light level). In a noise-free environment, there is no clear reason to choose one strategy over the other. We thus asked for what noise characteristics a clock performs better than an hourglass. We found that true clocks are always preferable when the external environment is the dominant source of noise[11]. On the other hand, when noise internal to the clock circuit is larger, there is no universal answer to the clock versus hourglass conundrum, but hourglasses can be better for specific choices of nonlinearities. One can also consider how best to design the clock itself in the face of internal noise. Specifically, we determined what response to light allows the clock to remain locked to the Earth's 24 hour cycle while providing maximal information about the time of day; it turns out that the optimal phase-response curve depends non-monotonically on the strength of the noise[10].

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