When cell biology meets theory

60 years of JCB

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Cell biologists now have tools and knowledge to generate useful quantitative data. But how can we make sense of these data, and are we measuring the correct parameters? Moreover, how can we test hypotheses quantitatively? To answer these questions, the theory of physics is required and is essential to the future of quantitative cell biology.

The study of complex biological systems requires a strong effort to give a detailed description of the components and of their interactions. For the past decades, the task of dissecting cellular mechanisms to identify proteins, their functions, and their partners has been the focus of cell biologists, with great success. For processes such as membrane trafficking or cell motility, we now have a close to exhaustive list of proteins involved, with more or less an idea of their function, localization, and partners. But do we really understand how the cell machinery works? Have we mastered the essential properties and control parameters to a point that would allow us to tune the cell system in a way that we determine? In some cases, yes, but this is mostly limited to a few concrete examples.

Part of the problem is that our description of cellular processes is poorly quantitative. For example, to be able to "tune" the cell, we would need not only to know which factors activate a signaling pathway, but to have an idea of the dose–response curve. And in order to understand which components are essential to establish such dependence, we would need to have an idea of the binding constants of all proteins in the pathway (phosphatase rates, kinase activity, etc.), a fairly unrealistic task when applied to all cellular processes. Does this mean we should not think of measuring these parameters? On the contrary, to understand how cells behave and how they react to their environment, we must be able to measure them. But what should be measured? Does every interaction, every activity, need to be measured?

Physicists have faced the same problem; i.e., wanting a quantitative understanding of matter in order to use it in a constructive way. However, as the nature of molecules and atoms was not known in the early 19th century, engineers and scientists used a macroscopic approach, measuring the correlative curve between macroscopic parameters (heat, temperature, dilatation, force, work, etc.). From these experimental data, they tried to determine the mathematical functions linking these parameters together, and established the classical laws of thermodynamics with almost no microscopic understanding of matter.

Once molecules and atoms had been discovered, and their interactions began to be explored, physicists faced another question: how do the microscopic structure of matter and its interactions lead to macroscopic thermodynamics laws? In a first approach, physicists believed that the only way to understand this issue was to measure the position and the speed of each molecule/atom and their interactions in the system. They soon realized that this was experimentally impossible, and it still is today. In fact, it turned out that theoretical tools that capitalize on statistical methods allowed physicists to describe accurately the behavior of gases, metals, and other material by measuring a few properties of their particles and then describing statistically the average behavior of the particles. The tools developed by statistical physics were able to predict emerging macroscopic properties (such as the expansion of a gas and electricity) from a small set of microscopic properties. The purpose of this article is to present how the same tools could play an essential role in understanding quantitatively how a cell works.

Why theory for cell biology?

We cell biologists value and have mastered a reductionist approach: the cell is such a complex system that understanding can only emerge from breaking its mechanisms into subparts and describing each of them in detail. In contrast, the physicist's strategy has two approaches: the first one is disregarding microscopic details, and focusing only on the macroscopic properties; and the second, bottom-up, approach is neglecting many microscopic details in order to focus on only some relevant interactions, then trying to explain the macroscopic properties by statistical analysis. Both methods have proven to be very useful for understanding biological systems. We will consider a few examples.

Theory can picture the behavior of cells without having any details about their internal regulation. A beautiful example is given in the work of Pascal Martin with Frank Jülicher (Hudspeth, 2008; Jülicher et al., 2009). Martin studies oscillations of the cilia of hair cells, which oscillate when sounds vibrate in the ear (Martin and Hudspeth, 1999). He observed spontaneous oscillations of the cilia in the absence of sound, and also measured a strong amplification of the oscillations triggered mechanically by attaching a vibrating glass fiber to the cilia (see Fig. 1). The amplification is much stronger for an extremely weak mechanical trigger, and absent when the fiber vibrates strongly. Theory explains these properties (Camalet et al., 2000): the hair cell is close to a Hopf bifurcation, a phenomenon in oscillatory dy-

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namics that describes a transition from a nonoscillatory state to a state where the system oscillates spontaneously. If the system is in a critical state close to the bifurcation, it does not oscillate spontaneously, but a very small stimulus will make it oscillate with a very strong response by crossing the bifurcation into the oscillatory mode (Martin et al., 2001). It also explains why cells in slightly nonoptimal conditions (small changes in ionic concentration, for example) will start to oscillate spontaneously as they cross the bifurcation. It may sound very theoretical, but it is not; this finding explains facts known to almost all of us. It explains why we can hear both very quiet and very loud sounds, because the ear is a nonlinear amplifier. It also explains why you hear a buzz after a loud concert, or why, in pathological tinnitus conditions, patients hear a noise (in fact generated by the ear) in a silent environment.

Theory can also predict the behavior of complex systems by greatly simplifying the molecular details. A prominent example is the description of biological networks by statistical approaches. The strategy is nicely presented in a book by Uri Alon (Alon, 2007). The essential concept in this description is to approximate the dose–response curve of a genetic or biochemical interaction by a fairly simple mathematical function, such as a sigmoidal curve, or even more simply, the step function. Activating interactions are modeled by a response = 0, which is below a threshold concentration of the activator, and by a response = 1, which is above. Inactivating interactions are the reverse. Surprisingly, this highly simplified model, which neglects the details of the dose–response curve of any single interaction within a node in the network, captures the response of nodes as a whole very well. A fascinating example is the incoherent feed-forward loop (IFFL, see Fig. 2), a network motif that can endow a system with the ability to compute fold change of an input (i.e., not the amount of the input molecule, but its quantitative change over time, i.e., its time derivative) and perfect adaptation (i.e., it detects the change and adapts when the input does not change anymore). A classical example of the IFFL is the galactose repression system in *E. coli* (see Fig. 2 B), which allows for dual control of gene expression with two inputs signals: when glucose is absent, the production of the galactose enzymes is much faster and shows a burst of activity compared to when glucose is present. What the example of the IFFL illustrates is that the response mostly depends on the architecture of the node, rather than the details of the biochemical or genetic interactions.

The book by Alon (2007) reviews a few beautiful examples of how, beyond the IFFL, when other network motifs are considered, other complex behaviors, such as transient activation or oscillatory activations, could be understood. Interestingly, a description of the architecture of a biological network remains experimentally attainable. Detailed knowledge about concentrations and specific activities are unnecessary and, therefore, omitted.

The message of these two examples is that theory achieves two important goals: (1) it simplifies the problems by weighting the effects of each component, and (2) it drastically reduces the numbers of parameters to consider. Moreover, in cases where your favorite cell is a black box, by measuring output variations correlated to input variations, theory can isolate an essential property that explains the relevant peculiar features of your cellular system, without knowing many molecular details. The two key questions are (1) which mathematical description and theory is the most appropriate? And (2) how do you build a fruitful collaboration with theoreticians?
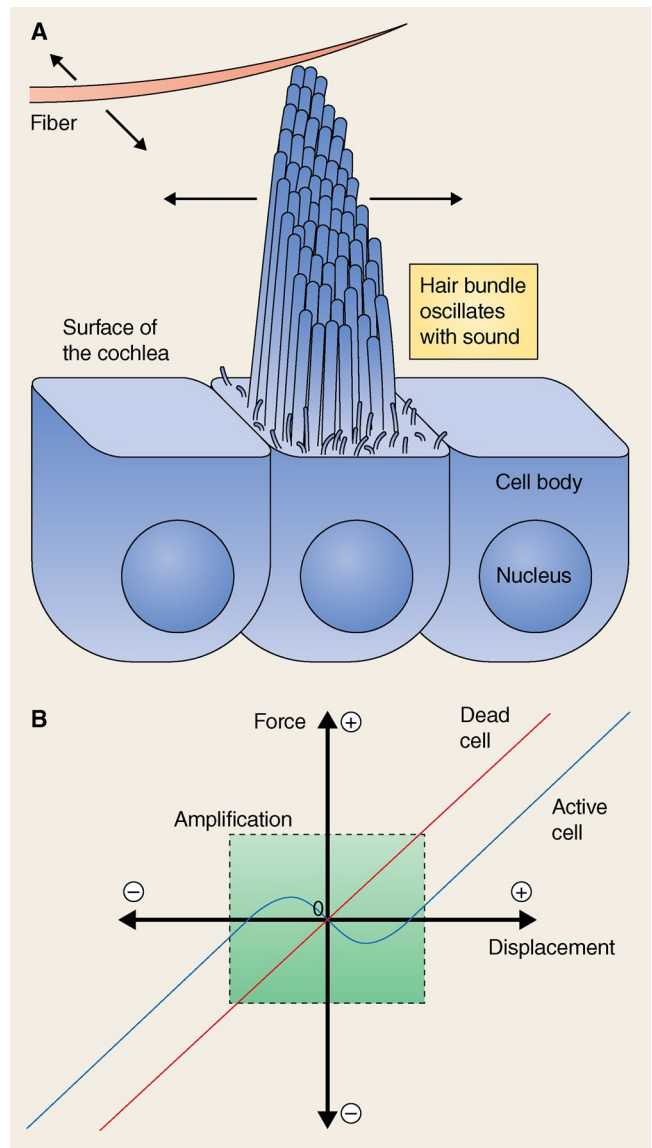
Figure 1. **Measuring the mechanics of the hair cell, the sound sensor of the ear.** (A) Schematic of the experimental setup. A glass fiber is glued on the ball-stick of the hair bundle sticking out of a hair cell. This fiber allows for the application of vibration to the bundle, and for the measurement of force from the bundle (by measuring the deflection of the fiber). (B) Typical curves of force with vibration amplitude (displacement) obtained from a dead cell (red) and from a living cell (blue). The red curve shows that the dead cell acts like a passive spring, as the force measured is linear with the vibration amplitude (Hook's law). In the green box, one can see a positive amplification of small vibration amplitudes in the living cell: for small positive displacements, the force measured by the fiber is negative (and the reverse for small negative displacements), showing that the cell actively moves its bundle in the direction of the movement applied by the fiber. The image is adapted from Tinevez et al. (2007).

Which theory for cell biologists?

Because cell and molecular biology is based to a large extent on our modern description of biochemical reactions, the most natural way to model cell processes is by using a set of differential equations that describes each of the known reactions that participate in the process. This is fairly easy to do if you are sure to have identified the essential partners and the most important phenomena in your system. A good example of this strategy is the model of lamellipodium protrusion (Mogilner and Edel-
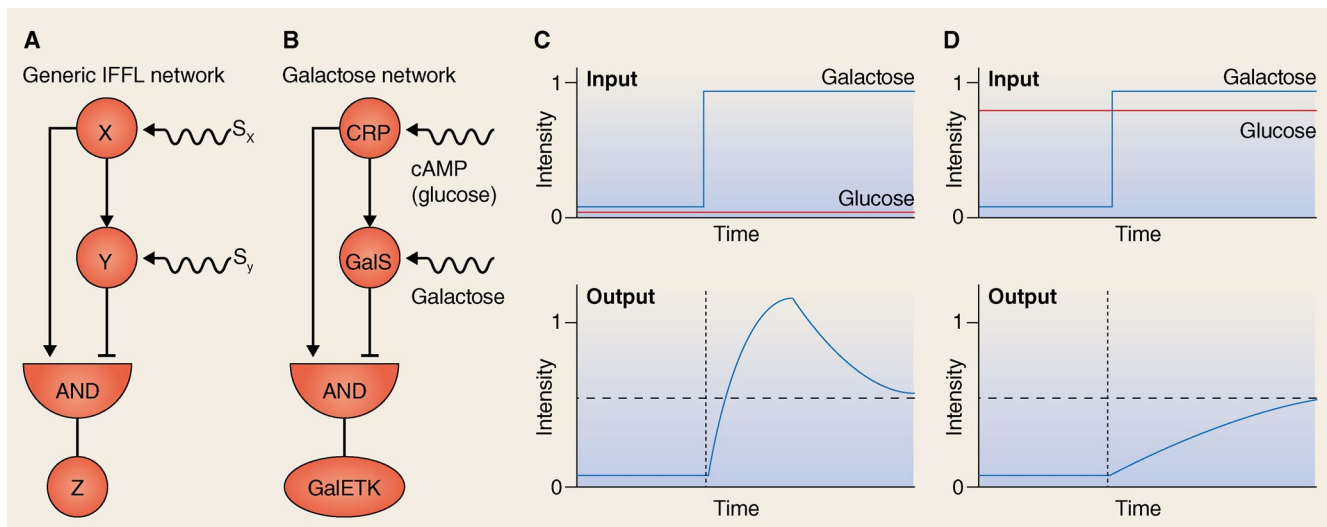
Figure 2. **The incoherent feed-forward loop (IFFL).** (A) General structure of an IFFL, with the two entries for signals (Sx and Sy). (B) The example of an IFFL found in the galactose genetic network of *E. coli*, working for the induction of enzymatic genes (GalETK for example) in the presence of galactose. CRP stands for cyclic AMP receptor protein, and is a catabolite gene activator of the Crp/Fnr transcriptional regulator family. GalS is a galactose-sensitive repressor that binds to the promoter of galactose-activated genes. (C) Typical response (i.e., transcription of *GalETK* genes) curve of the Galactose IFFL to a step addition of galactose in the absence of glucose. (D) Typical response of the Galactose IFFL to a step addition of galactose in the presence of glucose. Adapted from chapter 4.7 of Alon (2007).

stein-Keshet, 2002), based on biochemical interactions and reaction rates measured in vitro. In this case, many of the specific partners (WASP, Arp2/3, profilin, capping proteins, etc.) and parameters (concentrations, diffusion constants, growth rates of actin, etc.) have to be mathematically described, which implies that many equations need to be invoked, based on a myriad of "relevant" parameters. In the case of the lamellipodium, fortunately, many of the relevant parameters had been measured experimentally, which is already a tour-de-force, and others could be extracted from a fit of the theoretical model to experimental data. But the fits usually give large ranges of parameter values and low confidence that yield very little accuracy. The advantage of such a strategy is the ability to rely essentially on previous knowledge, obtained by standard cell biology and biochemistry tools, and to intrinsically account for dynamics. But at the end, it usually brings our understanding of the systems only a little forward: the only real gain if the model reproduces in silico the behavior of the system is to know that none of the essential parameters are missing. But how does one build a theory when the biological system is poorly described biochemically?

Another strategy is to consider the molecular system of interest at larger scales. For example, lamellipodia can be modeled as a two-dimensional hydrogel like agarose or gelatin. Flow dynamics and mechanical properties of polymer hydrogels under stress have been studied by soft-matter physicists, and laws have been established to describe their behavior. Two advantages of this strategy are: first, to reduce dramatically the number of parameters and equations to consider, making it much more reliably tested experimentally; and second, to isolate the essential properties of the systems that accounts for the behavior.

However, because biological systems have properties that are unique, they have to be taken into account in the theory. A good example where this approach has been very successful is active gel theory (for review see Prost et al., 2015), which aims at finding general equations of hydrogels made of cytoskeletal filaments and motors. This description contains the minimal, yet essential, properties of the cytoskeleton—polarized fila-

ments with dynamical growth, motors with contractile abilities (Kruse et al., 2005)—but the equations are similar to the ones used for nonbiological hydrogel flows. With this description, where the energy input is essentially described as an additional term corresponding to the work of an external force, scientists have been able to describe accurately the behavior of cells in various systems such as cell crawling (Kruse et al., 2006), actin waves and vortices observed in vitro and in vivo (Kruse et al., 2004), and many other cytoskeletal dynamical processes. For each specific system, the same set of properties is invoked, but not necessarily with the same importance.

However, there are two limits to this strategy; first, the parameters (flow, viscosity, diffusion, rigidity, force, and energy) that should be measured experimentally to validate the theory usually cannot be measured with standard biology tools. Second, it is difficult to estimate how much of the molecular details have to be taken into account in the macroscopic description, or how the specific molecular properties account for the macroscopic properties. The art of finding a useful theory thus relies on choosing the right level of microscopic complexity, and on coupling it to the macroscopic description. The right level of complexity is the one that captures the essence of biological behavior that wants to be understood by invoking a minimal set of physical and chemical phenomena. To know this a priori is not trivial and requires some trial and error as well as experience. Moreover, such theories require the integration of parameters from various origins and disciplines: biochemical parameters, such as the processivity of a motor, or a chemical rate of an enzyme, or physical parameters, such as the local density of ions or stiffness of cytoskeletal filaments. Seen from a theoretical point of view, the wide range of possible parameters to take into account requires the scientist to have knowledge in soft-matter physics, hydrodynamics, mechanics, physical chemistry, electrostatics, and, of course, statistical physics. The versatility of the researcher is thus an important component when building the theory and confronting it with a system of choice.

A fruitful way to overcome these limitations is to establish an intense discussion between biologists, experimental physicists, and theoreticians. This discussion is really at the heart of the interface between physicists and biologists. It is during this discussion that the important ingredients to understand a biological process will be selected. Most of the time, a constant back and forth discussion has to be organized, first to test qualitatively the effect of a proposed set of parameters in a few experiments and to isolate the most important ones. Then, the theory work provides quantitative descriptions that should be experimentally testable. Cell biology physics is one of the few fields of physics where experiments and theories addressing the same issues are performed at the same time, in the same environment.

Not surprisingly, many institutions where strong schools of physics of cell biology have been developed are where theory groups have been the drivers of interactions between scientists of various fields, and led to important discoveries. Some examples, though the list is hardly exhaustive: the Curie Institute in Paris, The Rockefeller University in New York, the Max-Planck Institutes of Cell Biology and Genetics, and for Physics of Complex Systems, in Dresden, the Weizmann Institute in Rehovot, Princeton University in New Jersey, and the National Center for Biological Sciences and Raman Institute in Bangalore. There, by establishing fruitful interactions with biologists, theoreticians were able to simplify some questions, and further conceptualize others. For example, they were able to sort out the roles of adhesive versus contractile forces in actin-mediated motility in various systems, such as *Listeria* (Bernheim-Grosswasser et al., 2002) and amoebae (Liu et al., 2015; Ruprecht et al., 2015), or the importance of active phase separation in compartmentalization of the cytosol (Brangwynne et al., 2009; Lee et al., 2013; Hyman et al., 2014). Because the theoretical tools can be applied to many biological systems that have to be studied experimentally with very different tools, it fostered comparisons between many systems, and resulted in more general findings than anticipated. As it can unify different fields of life sciences, and compare accurately and quickly between various biological systems, theory is an exceptional motor to discover general properties of biological systems.

How to convince a theoretician to interact with cell biologists

Another important, practical question is what aspects of biological systems are interesting challenges for a theoretician. The primary one is the fact that cells are open systems, which are then constantly brought out of equilibrium by energy input. This also means that cell biology phenomena are essentially driven by kinetics and dynamics, and not thermodynamics. Thus, hydrodynamics, diffusion, and chemical and biochemical rates rather than energy minimization are often the control parameters. Theoreticians are thus required to develop new tools in order to be able to approach this constantly changing state of living matter.

A good example of this is the emerging general theory of active gels (see the previous section and Prost et al., 2015). The active gel theory is based on hydrogel physics, but equations also account for an "active" term, which is usually a chemical potential accounting for the energy gain due to ATP hydrolysis. However, as hydrogel physics is essentially based on hydrodynamics, the active term takes the form of mechanical work (energy associated with a force and a displacement), which implies the imposition of a given transformation func-
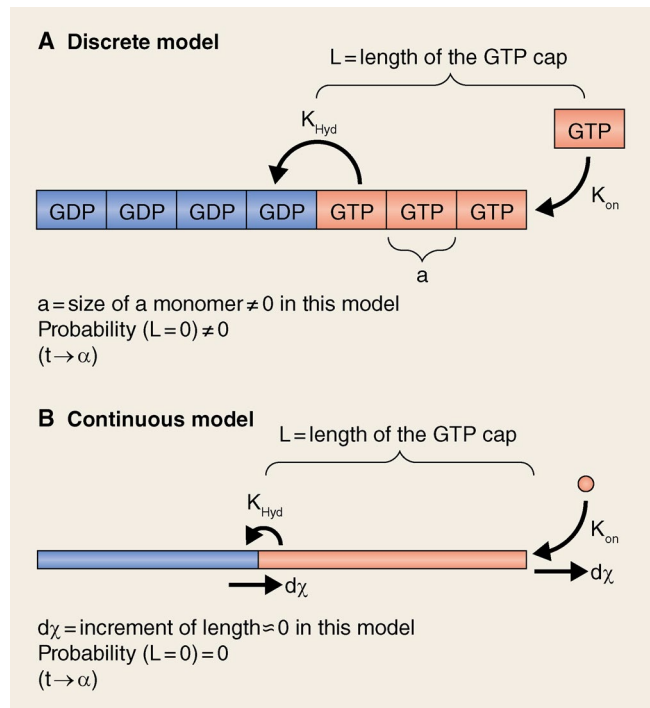

Figure 3. **Statistical mechanics of the microtubule GTP cap and catastrophe.** (A) In a case where the probability of having a GTP cap length of zero, which is when catastrophe would occur, is estimated from a discrete model, taking into account the molecular length of a dimer of tubulin, the probability is different from zero, thereby better predicting the observed rate of catastrophe. (B) In the case where the same probability is estimated from a continuous model, neglecting the size of the dimer, but allowing for use of integral calculations, the probability is zero, not describing accurately the dynamics of microtubules. See Flyvbjerg et al. (1994, 1996) for details.

tion for the energy of the ATP hydrolysis into the mechanical work. Even though the linear approximation for this function has been fairly good in describing experimental results, there is no general description for this "active" term, and it is thus still a challenge, when describing an active, biological system, to account for the energy input. A general description of "active" matter has yet to be found.

Another essential question to both biologists and physicists: How do macroscopic properties emerge from an assembly of molecules in interactions? In other words, how can we have an understanding of the biological processes through the many scales of biological organization? In the case of the cytoskeleton, for example, we now have a glimpse of a "bridging scale" understanding of cell motility. We are starting to understand how molecules auto-organize in a cell to make it crawl, and how cells crawl in development to form an organ. But this is far from being the same for all other cell processes, and the cell-to-tissue understanding is far less extended and quantitative than the molecular-to-cellular one.

Finally, a very interesting aspect of cells is their peculiar statistical properties. As explained in the introduction, statistical physics usually approximate the behavior of single particles to the average behavior, allowing for analytical treatment with continuous equations. The strength of this approach is to be able to understand how macroscopic properties emerge from microscopic features. Behind this strategy is the mathematical law that any statistical distribution can be averaged to a Gaussian

distribution for a large number (typically more than a few thousand). However, in biology, numbers of particles or molecules are often well below that, which means that even if the physics are the same, the behavior of the system will be different because of its low numbers. A striking example is given by a well-known property of microtubules: their length reduction through catastrophe. When the GTP cap is modeled through an analytical continuous probabilistic model, the probability of having a GTP cap of zero length is zero (Flyvbjerg et al., 1994, 1996), and the model thus cannot predict any rate of catastrophic events, which occurs when the GTP cap is zero (see Fig. 3). But when the model uses discrete numbers instead of analytical forms (sums instead of integrals), and includes the size of tubulin dimers, then with the same properties (a rate of polymerization of GTP-tubulin and a rate of GTP hydrolysis), the probability is not null. The model also accounts for the rate of catastrophe as a function of GTP concentration in a fairly quantitative manner.

Conclusion

The future of life sciences in general, and of cell biology in particular, is quantitative. Only a quantitative understanding allows us to accurately test hypothesis. In addition to being able to master biological processes for efficient biotechnology development, it is essential to describe them in quantitative detail. Theoretical and mathematical models will have a major impact in this quest, and we will all benefit, as cell biologists, from interacting more with theoreticians.

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