

Computational cell biology at the home of the helix

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The Computational Cell Biology Conference, held jointly by the Cold Spring Harbor Laboratory and the Wellcome Trust, was convened in the grand surroundings of Hinxtion Hall near Cambridge, UK. The high quality of the research presented at the meeting confirmed that the field of computational cell biology is maturing rapidly, which mirrors the progression of cell biology from being mostly descriptive to a more quantitative discipline.

There can be few more appropriate venues for a computational cell biology conference than the Francis Crick auditorium situated at the Sanger Centre in Hinxtion, Cambridge, UK. The conference, organized by Dennis Bray (U. Cambridge), Nicolas Le Novère (European Bioinformatics Institute) and Leslie Loew (U. Connecticut), demonstrated the extent to which computational approaches are becoming integral to research in cell biology. The field also continues to attract theoreticians who are making important contributions to mathematics as well as biology. In this respect, cell biology is beginning to resemble more closely the physical sciences with fruitful exchange between theory and experiment.

A recurring theme at the conference—and perhaps the central challenge for the field of systems biology—was the problem of complexity. In many biological systems, this complexity often translates into a large number of undetermined parameters. It is also possible that common modelling assumptions, such as treating the cell as a well-mixed reaction vessel, are not valid. It is for these reasons that the most profound insights into biological systems have so far been achieved through the judicious choice of model system.

Such systems typically contain a small number of components, and are both mathematically and experimentally tractable. An example of the success of this approach was provided by the work of keynote speaker Jim Ferrell (Stanford U.) on the cell cycle, which we discuss later in this report. Although these studies demonstrate clearly that



reductionism continues to be an extremely powerful approach for studying cell biology, it is not clear to what extent other problems—such as the immune system—can be reduced to a small set of key components or design principles. The future progress of the field therefore rests on identifying the key aspects of each system that can be tackled in isolation, or on developing new frameworks for dealing with uncertainty in biological systems. Another difficulty, which applies even to problems that are well defined, is that apparently contradictory conclusions can be reached from different modelling approaches. This situation arose in the work of Bela Mulder (FOM Institute for Atomic and Molecular Physics, Amsterdam) and Jun Allard (U. British Columbia, Vancouver) on plant microtubules, which we discuss in the final section.

Waving a clock inside a frog

An area that has seen the successful integration of theory and experiment is the cell cycle. Many of the discoveries in cell-cycle research have been made by studying

unicellular yeast species in which the cell cycle is controlled by a complex network that integrates signals both from within the cell and from the surrounding environment. The giant African frog *Xenopus laevis*, which lacks genetics tools and must choreograph mitosis with the development of a metazoan body plan, would at first glance appear to be an unpromising organism for gaining quantitative insight into the cell cycle. However, it is in this system that Ferrell and colleagues have produced fascinating work on the key components of the eukaryotic cell-cycle network.

A feature of *X. laevis* that makes it particularly amenable to quantitative studies is that the first 12 cleavages of the early embryo occur synchronously and at regular intervals of around half an hour. These blastomere cells seem to lack most, if not all, of the checkpoints and related signalling mechanisms present in other eukaryotic cells. There are therefore two main requirements for cell-cycle regulation in this system. The first is that the embryo should generate regular and robust clock-like signals for the cells to enter mitosis. The intervals between these signals must be controlled precisely to allow the cleavages to occur rapidly but with sufficient time for DNA synthesis to be completed. The second requirement is that this signal should be propagated quickly (in the order of minutes) across the extreme distances (by cellular scales) of around a millimetre that are present in the *X. laevis* embryo.

The basic timing mechanism will be familiar to anyone who has used an hour-glass; cyclin protein is synthesized at a

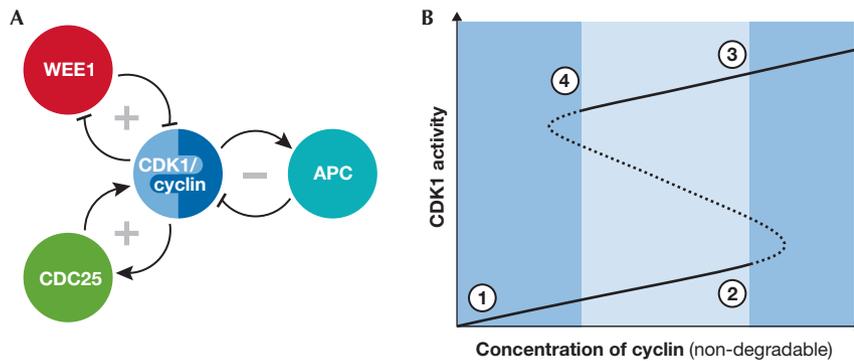


Fig 1 | The CDK1–cyclin regulatory network. (A) The network includes a negative feedback loop involving the anaphase-promoting complex (APC) and two positive feedback loops. The WEE1 loop inhibits CDK1 and the CDC25 loop promotes activation of CDK1. (B) The two positive feedback loops can be studied in isolation by adding a form of cyclin that cannot be degraded by the APC. In this situation, CDK1 displays either bistable (light blue) or monostable (dark blue) activity states. Solid and dotted lines represent stable and unstable equilibria, respectively. At low cyclin concentrations (1), WEE1 inhibits CDK1, CDC25 is inactive and CDK1 activity is low. At higher cyclin concentrations, two stable levels of CDK1 activity exist, a property known as bistability. However, the presence of the system in either of these stable states is dependent on the previous state of the system (hysteresis). Consequently CDK1 remains inactive. At a threshold cyclin concentration (2), the system becomes monostable as WEE1 inhibition is removed and CDC25 is activated. The system then changes rapidly to a stable equilibrium with high CDK1 activity (3). CDK1 activity will remain high until cyclin levels are reduced sufficiently for the left-hand monostable solution (4) to be reached.

constant rate, and its concentration therefore measures the time that the embryonic cells have spent in S phase. However, this smooth signal must be converted into a binary all-or-none response to ensure a sharp transition from S phase to mitosis and also to reset the cyclin concentration to basal levels at the start of the next round of DNA synthesis. The binary readout of cyclin levels is the activity of the cyclin-dependent kinase CDK1. On reaching a threshold concentration, the CDK1–cyclin complex activates the anaphase-promoting complex, which then degrades cyclin to reset the cell-cycle clock back to zero in a negative feedback loop. However, there are two additional positive feedback loops involving the mutual activation of CDK1 by the mitosis-promoting phosphatase CDC25, and its mutual inhibition by the mitosis-inhibiting kinase WEE1 (Fig 1A). Ferrell presented data showing that these feedback loops are highly cooperative, which leads to more step-like responses of both WEE1 and particularly CDC25 to CDK1 activity (Ferrell, 2009). The combined action of these two regulators of CDK1 leads to bistability and hysteresis in CDK1 activation, thus making the commitment of the embryo to a new cell-cycle state both decisive and irreversible over short timescales (Fig 1B).

In the second half of his talk, Ferrell addressed how these signals are propagated in the embryo. It is well known that the time for diffusion to transport proteins in the cell scales quadratically with increasing distances. Consequently, a typical protein would take tens of hours to diffuse across the *X. laevis* embryo, a time that is inconsistent with a division cycle of around 30 min. To investigate this, Ferrell showed an experiment that uses the other convenient feature of *X. laevis* embryos: their ability to complete the early rounds of mitosis as a cell-free extract. One such cell extract was supplemented with a green fluorescent protein (GFP) marker containing a nuclear localization signal and placed in a linear microfluidic channel. This marker could then be used to visualize mitotic entry and exit by the formation and breakdown of GFP-labelled nuclei. This experiment showed that the signal for nuclear envelope breakdown is transmitted as a wave that travels between nuclei at a rate faster than even the most rapidly diffusing proteins. This suggests a molecular mechanism whereby a localized source of activated CDK1 is transported outwards by diffusion over short distances: these activated CDK1 molecules phosphorylate both WEE1 and

CDC25, which in turn diffuse further outwards leading to activation of additional CDK1 molecules. This process is repeated and thus leads to an expanding wave front of CDK1 activity. One of the roles of cyclin in this spatial model is therefore to control how permissive the cytoplasm is to the transmission of waves of activated CDK1. In the future, it will be interesting to uncover how these cytoplasmic signals are transduced into a binary mitotic state in the nucleus, and how synchronization is achieved in the complex three-dimensional environment of intact embryos.

Taming unruly microtubules

One of the fundamental building blocks of tissue morphogenesis is polarized cellular growth. The cytoskeleton is essential for cell polarity in both plant and animal tissues. However, the presence of a rigid cellulose wall in plant cells leads to a cytoskeletal organization that is very different to that observed in animal cells. Higher plants lack centrosomes and most microtubule nucleation occurs at the cortex of interphase cells. The microtubules in elongating plant cells are oriented perpendicularly to the axis of cellular growth, and this alignment is believed to localize the exocytic machinery and provide tracks for the oriented deposition of cellulose microfibrils in the cell wall. The question, addressed separately by Mulder and Allard, is how the cell generates oriented microtubule arrays on the plant cell cortex.

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Observations by Dixit & Cyr (2003) and others have shown that microtubules nucleated at the plant cell cortex are stationary and that their dynamics are determined by growth and shrinkage of the microtubule ends. Plant microtubules undergo dynamic instability, which means that they switch abruptly from growing to shrinking states in a process known as ‘catastrophe’, and also make the opposite transition from shrinkage to growth in a process known as ‘rescue’. As plant microtubules are confined to the two-dimensional geometry of the cell cortex, growing microtubule plus-ends frequently encounter stationary microtubules

in their path. When this occurs there are three possible outcomes. The first possibility is that the growing microtubule is unaffected by the stationary microtubule and continues growth in the same direction. The second possibility is that the collision induces a catastrophe in the growing microtubule. Finally, the microtubule might continue to grow, but in a direction that is aligned with the stationary microtubule in a process known as 'zippering'. The likelihood of each outcome is dependent on the contact angle of the two microtubules, with zippering occurring frequently at shallow angles and the other two outcomes prevalent at steeper angles.

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In the work of Mulder and colleagues (Tindemans *et al*, 2010), these processes were modelled using stochastic simulations and mean field theory. The two approaches are complementary, as simulations consider the behaviour of individual microtubules, whereas in the theory these details are neglected and the system is instead modelled with coupled differential equations representing the density of microtubules in a particular orientation. To a layperson, it would seem surprising that the organization of fibres can be described by using an average across the spatial domain; however, this approach is the basis of some of the most elegant and successful theories in polymer physics. The predictions from Mulder's model of plant microtubules are in impressive agreement with the simulations and exemplify the advantages of a

successful theory over simulation: first, theories can be used to succinctly describe a large number of situations, and second, they can often be used to identify the essential features of a system. In this case, the different aspects of microtubule growth are shown to influence the system through a single control parameter.

The theory and simulations were then used to identify which features are important for ordering of the microtubule array. This showed that collision-induced catastrophes are essential for generating ordered arrays but that zippering has little effect. The intuitive explanation for this type of ordering is that collision-induced catastrophes lead to selective retention of microtubules with a specific orientation. Ordering then arises from a system containing randomly oriented microtubules when a local region, by chance, accumulates an array of aligned microtubules. This array causes the catastrophe and eventually destruction of microtubules that encounter it with a different orientation. Microtubules that are nucleated with the same orientation further reinforce this effect until all of the long microtubules in the system eventually become aligned.

The converse conclusion was reached by Allard and colleagues (Allard *et al*, 2010), in a study using a similar type of stochastic simulation, which showed that zippering is essential for microtubule alignment but that collision-induced catastrophes are less important. The most probable reason for the discrepancy between the two studies is the choice of parameters used to model the system. As microtubules alternate between periods of growth and shrinkage, their length, in the absence of other effects, depends on the difference between the average length added during a growing cycle and that removed during a shrinking cycle. If this quantity is negative

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then the microtubules have a finite length. A positive value leads to unbounded growth. In most of Allard's models, the microtubules are nucleated at a low rate but exist in the unbounded growth regime, or are long compared with the cell surface. In this regime, collision-induced catastrophes are less effective at microtubule alignment, as the induction of catastrophe is less likely to cause the microtubule to completely depolymerize. Intuitively, zippering could induce ordering if several aligned or zippered microtubules successfully self-intersect across a periodic boundary. This array would then force microtubules interacting with it at steep angles to cease growth, while zippering those with a similar orientation.

As the two studies use experimental data from different plant species, it is possible that either or both mechanisms could be important under different conditions. However, a full resolution of the paradox requires further experiments to perturb zippering or microtubule growth, or perhaps to measure additional features of the system such as the dwell time of tubulin subunits at the plant cortex.

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