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Review

Simple, realistic models of complex biological processes: Positive feedback and bistability in a cell fate switch and a cell cycle oscillator

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ABSTRACT

Here we review some of our work over the last decade on *Xenopus* oocyte maturation, a cell fate switch, and the *Xenopus* embryonic cell cycle, a highly dynamical process. Our approach has been to start with wiring diagrams for the regulatory networks that underpin the processes; carry out quantitative experiments to describe the response functions for individual legs of the networks; and then construct simple analytical models based on chemical kinetic theory and the graphical rate-balance formalism. These studies support the view that the all-or-none, irreversible nature of oocyte maturation arises from a saddle–node bifurcation in the regulatory system that drives the process, and that the clock-like oscillations of the embryo are built upon a hysteretic switch with two saddle–node bifurcations. We believe that this type of reductionistic systems biology holds great promise for understanding complicated biochemical processes in simpler terms.

1. Introduction

The South African clawed frog *Xenopus laevis* provides two powerful experimental systems for the study of the regulation of the universal M-phase trigger, CDK1: the maturation of *Xenopus* oocytes, and the rapid embryonic cell cycle [1]. Oocyte maturation is essentially a cell fate induction process, with the cell switching between two fairly static states, the G2-arrested immature oocyte and the M2-arrested mature oocyte. The embryonic cell cycle, in contrast, is dynamic. There are no steady states, and the embryo does not pause even in the presence of DNA-damaging agents. But both oocyte maturation and the embryonic cell cycle are well-suited to the detailed, quantitative biochemical dissection of the regulatory networks that drive these processes. And in both cases, our understanding of the processes has depended not only on experiments, but also on modeling studies that provide tests of whether our basic view of these processes is tenable or not, and on the theory of non-linear dynamics, which provides a more abstract and simpler view of the essence of this biology.

Here we review some of our work over the last decade on *Xenopus* oocyte maturation and the embryonic cell cycle. Our approach has been to start with wiring diagrams for the regulatory networks that underpin the processes; carry out quantitative experiments to describe the response functions for individual legs of the networks. And then construct simple analytical models based on chemical kinetic theory and the graphical rate-balance formalism. These studies support the view that the all-or-none, irreversible nature of oocyte maturation arises from a saddle–node bifurcation in the regulatory system that drives the process, and that the clock-like oscillations of the embryo are built upon a hysteretic switch with two saddle–node bifurcations. In a sense, oocyte maturation is as easy as falling off a log, and the embryonic cell cycle is a lot like falling off two logs over and over again.

2. Positive feedback, bistability, and oocyte maturation

2.1. Xenopus oocyte maturation as a cell fate switch

Xenopus oocytes begin life as cells not much larger than typical somatic cells (Fig. 1). They go through a normal G1-phase and a normal S-phase, and then carry out the early events of meiotic prophase: their homologous chromosomes pair up and undergo recombination. But instead of immediately proceeding to the first meiotic division, the oocyte enters a several-month-long growth phase. It grows to about the volume (1 μL) and protein content (25 μg) of approximately 400 000 NIH 3T3 cells, and then it stops. At this point the cell is technically still in meiotic prophase, but for

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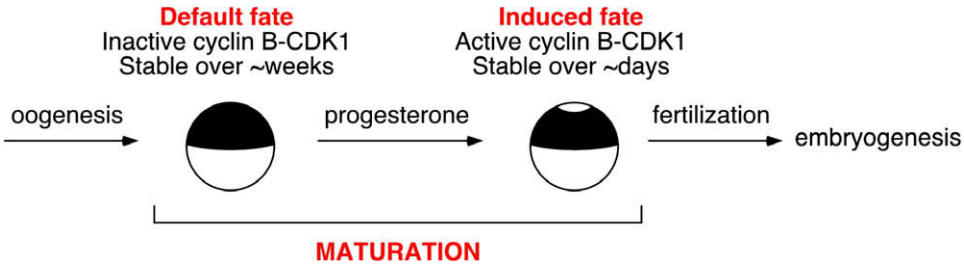

Fig. 1. *Xenopus* oocyte maturation as a switch between two cell fates.

practical purposes can be considered to be in G2-phase—transcription is taking place and the M-phase cyclins are present but locked in inactive complexes with CDK1—and its default fate is to remain arrested indefinitely in this state, with all its various opposing processes (protein synthesis/degradation, phosphorylation/dephosphorylation, anabolism/catabolism, etc.) in balance.

In response to gonadotropins released from the frog pituitary, the ovarian epithelial cells surrounding the oocyte release maturation-promoting hormones, which cause the immature oocyte to resume the process of meiosis. The classical maturation-inducing hormone is progesterone, and *Xenopus* oocytes possess both classical progesterone receptors [2–5] and seven-transmembrane G-protein-coupled progesterone receptors [6]. However, progesterone undergoes metabolism in the oocyte, and there is evidence that androgens and androgen receptors may ultimately mediate progesterone's effects [7]. Regardless of whether a progestin or an androgen is the ultimate trigger, the effects of progesterone on immature oocytes are striking. The oocyte leaves its G2-arrest state, carries out the first asymmetrical meiotic division, enters meiosis 2, and then arrests in metaphase of meiosis 2. This progression from the G2-arrest state to the meiosis 2-arrest state is termed maturation. After maturation the oocyte is ovulated, acquires a jelly coat, and is laid by the frog. It then drifts in the pond in this arrested state until either it is fertilized, which allows it to complete meiosis and commence embryogenesis, or it undergoes apoptosis.

In some respects oocyte maturation is an unusual example of a cell fate switch. Transcription is not absolutely required, and at least some aspects of maturation can even proceed in an enucleated oocyte [8]. However, in other respects it is absolutely typical: the cell responds to an external trigger by undergoing an all-or-none, irreversible change in its appearance, its biochemical state, and its developmental potential.

2.2. Mos, p42 MAPK, and CDK1 activation

Although many details remain to be worked out, in broad outline the signaling network that mediates progesterone-induced oocyte maturation is well-understood (Fig. 2). Progesterone stimulates the translation of the Mos oncoprotein, a MAP kinase kinase kinase (MAPKKK). Active Mos phosphorylates and activates the MAPKK MEK1, which then phosphorylates and activates ERK2 (which in *Xenopus* is often called p42 MAPK). Inhibitors of these MAPK cascade proteins inhibit oocyte maturation, and activated forms of the proteins can initiate maturation in the absence of progesterone.[1] p42 MAPK activation then brings about the dephosphorylation and activation of cyclin B-CDK1 complexes (sometimes termed "latent MPF", for latent maturation-promoting

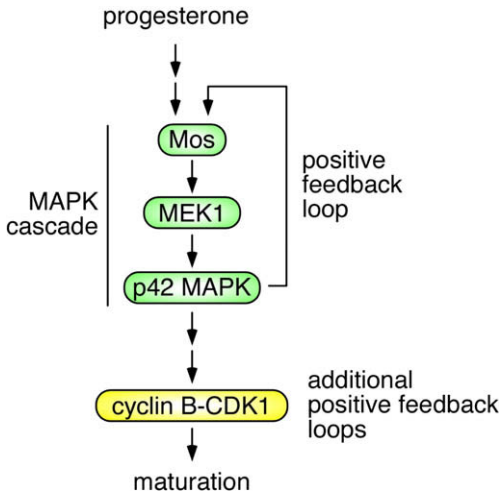

Fig. 2. Signal transduction pathways involved in *Xenopus* oocyte maturation.

factor, or "pre-MPF"). Activated cyclin B-CDK1 complexes then cause the oocyte to re-enter meiotic M-phase.

Cyclin–CDK complexes are thought to function as near perfect switches at the level of the individual complex: for example, a fully activated cyclin A-CDK2 complex is approximately 10^9-fold more active than an "inactive" CDK2 monomer [9]. But an oocyte possesses $\sim 10^{10}$ cyclin B-CDK1 complexes, meaning that even if an individual complex is perfectly all-or-none in its activity state, the population of cyclin B-CDK1 complexes could, in principle, settle into a nearly continuous range of graded activities. This raises the question of how the all-or-none character of oocyte maturation arises. Is the process all-or-none at the level of p42 MAPK activation and/or CDK1 activation? And how do these reversible activation processes culminate in an irreversible cell fate change?

2.3. The all-or-none, irreversible response depends upon positive feedback

Analysis of individual oocytes treated with various concentrations of progesterone demonstrated that the steady-state response of the oocyte's MAPK cascade is essentially all-or-none (Fig. 3). At intermediate concentrations of progesterone, individual oocytes were found to have either all of their p42 MAPK non-phosphorylated or all of it phosphorylated. Thus, somewhere between the progesterone receptor and the bottom of the MAPK cascade, a graded "analog" progesterone stimulus is converted to a "digital" MAPK response. Moreover, the steady-state response of MAPK to microinjected Mos is also all-or-none [10]. This demonstrates that the MAPK cascade can generate an all-or-none response, not simply propagate one. A plausible mechanism for the generation of the all-or-none response was suggested by the discovery that, in oocytes, p42 MAPK and CDK1 are organized in positive feedback

[1] There is some disagreement on whether the Mos/MEK/MAPK cascade is required or dispensable for progesterone-induced oocyte maturation. See for example, Refs. [9–11].

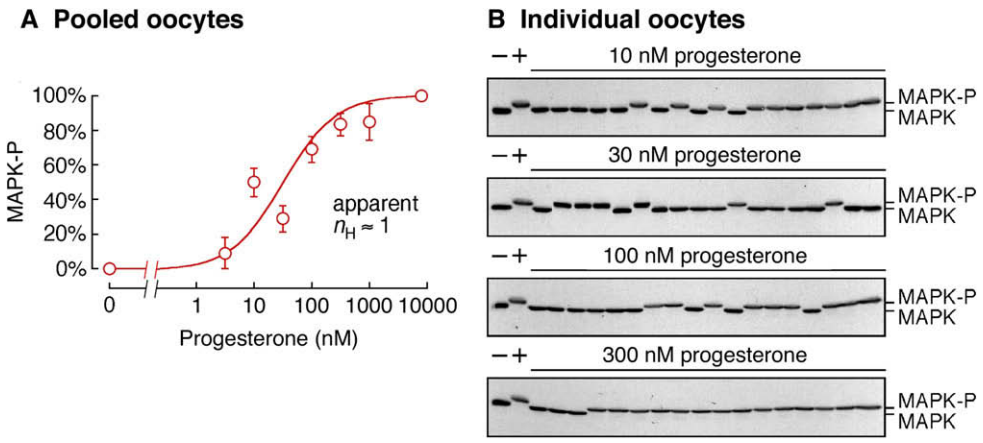

Fig. 3. The steady-state response of p42 MAPK to progesterone in oocytes. (A) When oocytes are pooled, the response to progesterone appears to be graded, with an apparent Hill coefficient of about 1. (B) When individual oocytes are examined (a single oocyte is large enough to easily provide enough protein for an immunoblot; right), the response is all-or-none. The lanes denoted – and + are control samples of immature and mature oocytes, respectively. Adapted from [10].

loops. Mos stimulates p42 MAPK activation, but p42 MAPK activation also feeds back to stimulate Mos synthesis [11–13]. This is shown schematically in Fig. 2.

Under conditions where the positive feedback is blocked, microinjected Mos can still induce oocyte maturation, but the p42 MAPK response is no longer all-or-none in character [10]. This demonstrates that positive feedback is required for the all-or-none response.

In addition, the response of oocytes to progesterone is normally irreversible; after a few hours of incubation with progesterone, the oocyte becomes irrevocably committed to maturation, and will remain a mature oocyte with active p42 MAPK and active CDK1 for many hours after the progesterone is washed away. Positive feedback is required for this irreversibility. When the positive feedback is compromised, the activation of p42 MAPK and CDK1 becomes reversible [14]. Thus, positive feedback is required for both the all-or-none character of the process and the irreversibility of it.

2.4. A saddle–node bifurcation in the dynamics of the Mos/MAPK cascade explains the all-or-none, irreversible response

When p42 MAPK is fully active and fully phosphorylated, its phosphates are still cycling on and off with a dephosphorylation half-time of a few minutes [15]. Thus in mature oocytes the p42 MAPK is actively maintained in a phosphorylated steady state rather than kinetically stuck there. How then does positive feedback convert the graded, reversible activation of p42 MAPK into an all-or-none, irreversible steady-state response? A plausible explanation can be derived from chemical kinetics and the theory of non-linear dynamics. We will begin by examining under what conditions the Mos/MAPK system can be in a steady state, like the unchanging low p42 MAPK-activity state of the immature oocyte or the high p42 MAPK-activity state of the mature oocyte.

At steady state, the rate of Mos production must equal the rate of Mos destruction. We assume that Mos degradation is a simple first-order process:

$$\textit{Degradation rate} = k_{dest}Mos \tag{1}$$

There is one undetermined parameter here, k_{dest}; we have taken it to be equal to 1.

Next we assume Mos synthesis consists of both a basal, progesterone (*prog*) dependent rate and a positive feedback contribution to the rate. The positive feedback probably depends both directly on the level of p42 MAPK activity and indirectly on p42 MAPK through the intermediacy of proteins like CDK1. For simplicity we will assume the strength of the feedback is a linear function of the MAPK activity:

$$\textit{Synthesis rate} = k_{basal}prog + k_{feedback}MAPK^{*} \tag{2}$$

where $MAPK^{*}$ denotes the concentration of active p42 MAPK. Biochemical studies in *Xenopus* extracts have established that the activation of p42 MAPK by Mos is highly ultrasensitive; that is, the response resembles that of a highly cooperative enzyme [16]. Empirically, the response is well approximated by a Hill function with a Hill coefficient of 5. Assuming that MAPK activation comes to equilibrium rapidly compared to Mos synthesis and degradation, we can write:

$$\textit{Synthesis rate} = k_{basal}prog + f_{feedback}\frac{Mos^{n}}{EC50^{n} + Mos^{n}} \tag{3}$$

where $f_{feedback} = k_{feedback}MAPK_{tot}$, the Hill coefficient $n = 5$, and the $EC50$ is approximately 20 nM [16]. There are two undetermined parameters in Eq. (3): $f_{feedback}$ and k_{basal}. If we are content to measure *prog* in relative rather than absolute terms, we can arbitrarily choose a value for k_{basal}; here we will assume $k_{basal} = 0.2$, which makes the progesterone concentration come out in nM units. This leaves one undetermined parameter, $f_{feedback}$, which we have taken here to be 40.

We can now plot the synthesis rate (for various assumed values of *prog*) and the degradation rate as functions of the Mos concentration. Where the curves intersect, the system is in steady state—the synthesis rate balances the degradation rate—and the Mos concentrations at which the intersections occur are the possible steady-state levels of Mos for a given concentration of progesterone. Fig. 4 shows Mos's degradation rate as a function of the Mos concentration in blue and Mos's synthesis rate as a function of the Mos concentration (for three concentrations of progesterone) in red. Because of the Hill function built into Mos's synthesis rate, the synthesis rate can wrap around the degradation rate and the curves can intersect in three places. This means that, at some (low) progesterone concentrations, the Mos/MAPK system can have three steady states. The first and third are stable, because a small deviation in the Mos concentration will shift the balance of Mos synthesis and degradation in a way that returns the system towards the steady state. The middle steady state represents an unstable threshold.

We can also derive an analytical expression for the steady-state concentration of Mos as an implicit function of the concentration of progesterone:

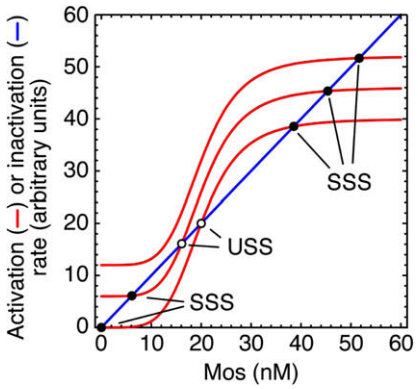

Fig. 4. Rate-balance analysis of Mos synthesis and degradation in oocytes treated with progesterone. The curves are given by Eqs. (1) and (3). The lowest activation curve (red) corresponds to zero progesterone. The middle curve represents a sub-threshold concentration of progesterone (30 nM). The upper curve represents a supra-threshold concentration of progesterone (60 nM). A slightly more complex version of this model and this analysis was presented in [10].

$$prog = \frac{k_{dest}EC50^n Mos - \frac{f_{feedback}}{k_{dest}} Mos^n + k_{dest} Mos^{n+1}}{k_{basal}(EC50^n + Mos^n)} \quad (4)$$

Eq. (4) is plotted in Fig. 5, taking the values for the various parameters as described above. The relationship between the output (Mos) and the input (*prog*) is not the usual Michaelian hyperbolic one, and not a sigmoidal cooperative or ultrasensitive one, but rather an S-shaped relationship. At low progesterone concentrations, the system has a stable steady state (the off-state) and an unstable threshold. As the progesterone rises, the off-state approaches the threshold. At a critical progesterone concentration the off-state and the threshold meet and annihilate each other at what is often termed a saddle–node bifurcation.[2] Above this progesterone concentration, the system has no choice but to fall "up" to a state with a substantially higher Mos concentration. The traversal of this saddle–node bifurcation explains the all-or-none character of Mos/MAPK activation in oocytes. It also explains the irreversibility; once the system has made it to the on-state, one can remove the progesterone and the positive feedback maintains the system in the on-state indefinitely.

In summary, single-cell, quantitative experiments demonstrated that the all-or-none, irreversible process of *Xenopus* oocyte maturation is driven by a complex regulatory system that generates an all-or-none, irreversible biochemical response. In essence, the system functions like a toggle switch flipping between two discrete alternative cellular states. The key ingredients of the system include positive feedback and a highly ultrasensitive response within the positive feedback loop Modeling can account for this behavior, and the theory of non-linear dynamics explains both the all-or-none character and the irreversibility of the response as arising from a dynamical system with a saddle–node bifurcation. The combination of quantitative experiments, modeling, and theory allows the events to be not just described but also understood.

More than 40 years ago, Monod and Jacob asserted in a much-cited, highly influential paper, that the differentiated state was maintained by gene circuits that constituted double-negative feedback loops [17]. Although some of the details here are different from those imagined by Monod and Jacob—the key regulation is translational and post-translational rather than transcriptional, and the feedback loops include positive feedback loops, not just double-negative feedback loops—the essential concepts of the system that drives oocyte maturation fit well with their ideas. The differentiated state is actively maintained by a non-linear dynamical regulatory system; the immature and mature oocytes correspond to two stable steady states of the regulatory system; and the process of maturation corresponds to the traversal of a bifurcation.

More recently, synthetic biology experiments have demonstrated that artificial gene regulatory circuits similar to the systems envisioned by Monod and Jacob and to the oocyte's Mos/MAPK system can function as bistable toggle switches [18,19], supporting the notion that if you understand a biological circuit, you will be able to exploit its design for other purposes.

3. Positive feedback, bistability, and the mitotic trigger

The cell cycle is often regarded as a complex but orderly sequence of contingent events: the cell grows in G1-phase, then once it grows to a sufficient size carries out DNA replication; once DNA replication is complete, it enters mitosis; and once the chromosomes are lined up properly in mitosis, it carries out sister chromatid separation and mitotic exit [20,21]. Murray and Kirschner dubbed this the "dominoes" view of the cell cycle [22]. However, some cell cycles do not behave this way. Notably, the well-studied embryonic cell cycle of the frog *X. laevis* behaves more like a clock or an autonomous oscillator than a sequence of dominoes. One can block the endpoints of the cell cycle—DNA replication or cell division—and still have the biochemical pulse of the cell cycle, the periodic activation and inactivation of CDK1, proceed fairly normally. Autonomous oscillators arise in a variety of biological contexts, ranging from the one-per-second action potentials of the sino-atrial node, through the one-per-day transcriptional cycles of the circadian rhythm, to the monthly and yearly cycles of the endocrine system. The powerful experimental approaches available in *Xenopus* embryos and cell free extracts meant that it might be possible to biochemically determine how this particular autonomous oscillator works.

The basic circuitry of the embryonic cell cycle oscillator is shown in Fig. 6. Mitotic cyclins (including cyclin A1, two different cyclin B1 gene products, cyclin B2, cyclin B4, and cyclin B5) are synthesized at a roughly constant rate, and during interphase they

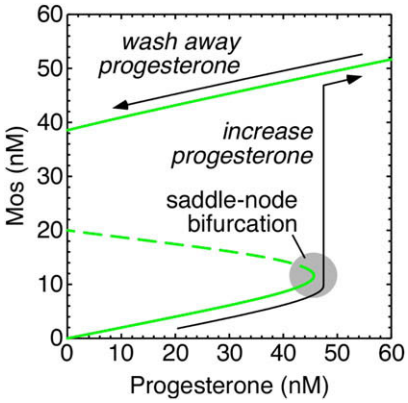

Fig. 5. Modeled steady-state response of a *Xenopus* to progesterone. The green curve is the steady-state response of Mos to progesterone as described by Eq. (4). The dashed portion of the curve represents an unstable threshold. The solid portions represent stable steady states. The process of oocyte maturation is viewed as the traversal of a saddle–node bifurcation. This explains the all-or-none character of maturation and the irreversibility of the process. Adapted from [10,14].

[2] The reason for calling this a saddle–node bifurcation is more obvious in systems with two time-dependent variables than it is in this case, where Mos is the only time-dependent variable. In a two-variable system, the middle steady-state is a saddle point in the two-dimensional vector field representation of the system. The term "node" is synonymous with "stable steady-state". So at a saddle–node bifurcation, a saddle and a node come together and annihilate each other. Or, from the other direction, a steady-state appears out of thin air and then immediately splits into a saddle and a node.

are stable. The cyclins quickly associate with CDK1, and when the cyclin–CDK1 complex is in the proper phosphorylation state (Thr 161 phosphorylated; Thr 14 and Tyr 15 dephosphorylated), the complex is active and phosphorylates (probably) hundreds of mitotic substrates (see [23,24]). One of the targets of active cyclin–CDK1 is the Cdc20-bound form of the anaphase-promoting complex (APC-Cdc20), an E3 ubiquityl ligase that carries out the polyubiquitylation of free and CDK1-bound cyclin proteins, which are then degraded by the proteasome. Cyclin–CDK1 and APC-Cdc20 thus constitute a negative feedback loop. This negative feedback is essential for cell cycle oscillations; if the cell cycle is driven by an N-terminal cyclin deletion mutant that is not efficiently recognized by APC-Cdc20, the result is a mitotic arrest [25].

In addition, the cyclin–CDK1 network includes a positive feedback loop and a double-negative feedback loop (Fig. 6). Active Wee1 can phosphorylate (at Tyr 15) CDK1 and inactivate it; conversely, CDK1 can phosphorylate Wee1 (at multiple sites) and inactivate it. Thus Wee1 and CDK1 are mutually antagonistic and share the double-negative relationship envisioned by Monod and Jacob as critical for the differentiated state. Active Cdc25 can dephosphorylated the site Wee1 phosphorylates in CDK1; conversely, CDK1 can phosphorylate Cdc25 (at multiple sites) and bring about its activation. Thus Cdc25 and CDK1 form a positive feedback loop. While negative feedback loops are essential for biological oscillations, it is possible to design oscillator models that lack positive feedback loops and yet still oscillate quite robustly. Examples of negative-feedback-only oscillators include the classical Goodwin oscillator [26], Albert Goldbeter's original model of the mitotic oscillator [27], and Michael Elowitz's synthetic Repressilator [28].

To test the importance of positive feedback for mitotic cycles, Pomerening et al. short-circuited the loops by supplementing the 200 nM CDK1 normally present in a *Xenopus* egg extract with 200 nM recombinant WT-CDK1 or 200 nM recombinant CDK1AF, a phosphorylation site mutant that cannot be inactivated by Wee1 and need not be activated by Cdc25. They found that this modest perturbation changed the oscillations in CDK1 from being explosive and sustained to more sinusoidal and damped (Fig. 7) [29]. The CDK1AF-treated extracts ultimately settled in a state that appeared to be intermediate between interphase and mitosis, as assessed by the morphology of sperm chromatin added as a reporter to the extract. Manipulations that forced the extract to rely exclusively on the CDK1AF protein rather than the endogenous CDK1 protein resulted in even more severe damping [29]. These results argue that the evolutionarily-conserved positive feedback and double-negative feedback loops are essential components of the mitotic oscillator.

3.1. The CDK1/Wee1/Cdc25 sub-circuit functions as a bistable mitotic trigger

If positive feedback is essential for sustained embryonic cell cycle oscillations, what exactly is it contributing to the oscillator?

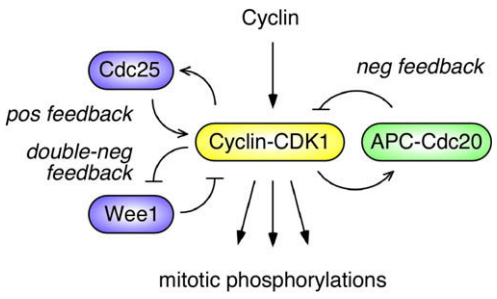

Fig. 6. Schematic view of the mitotic oscillator in *Xenopus* embryos and *Xenopus* egg extracts.

After all, as mentioned above, it is perfectly possible to build an oscillator circuit without positive feedback loops.

One plausible hypothesis is that the CDK1/Wee1/Cdc25 circuit, like the Mos/MAPK cascade in oocytes, is functioning as a bistable toggle switch. If so it would explain the longstanding observation of an apparent threshold in the response of CDK1 to non-degradable cyclin in interphase *Xenopus* egg extracts [30,31]: the threshold concentration of cyclin could be the point at which the system goes through a saddle–node bifurcation. To test this hypothesis, two groups carried out titration experiments to determine the steady-state response of CDK1 to non-degradable cyclin in extracts coming out of M-phase vs. coming out of interphase. The result was an S-shaped, hysteretic, stimulus-response loop (Fig. 8). At low concentrations of cyclin (<40 nM), only low levels of CDK1 activity were obtained. At high concentrations (>75 nM), only high levels of CDK1 activity were obtained. However, in between these concentrations, there were two possible steady-state levels of CDK1 activity (Fig. 8). This indicates that, between these two thresholds, the system was bistable. Bistability could also be seen by examination of reporter sperm chromatin added to the extract: over a range of ~40–75 nM cyclin, the sperm remained indefinitely in an interphase-like state if the extract was coming from interphase, but remained indefinitely in an M-phase-like state if the extract was coming from M-phase.

Thus, the CDK1/Cdc25/Wee1 system functions as a bistable trigger for mitosis, a possibility anticipated by Tyson and Novak [32] and Thron [33] not long after it was discovered that there was positive feedback in the system [31,34–36]. But, in contrast to the Mos/MAPK system in *Xenopus* oocytes, it generates a hysteretic response rather than an irreversible one.

3.2. Rationalizing the system's hysteretic response

In the same way that we used rate-balance analysis to analyze the steady states of the Mos/MAPK systems (Figs. 3 and 4), it should be possible to use a rate-balance analysis for the activation and inactivation of cyclin–CDK1 complexes in the absence of cyclin degradation, and determine how the hysteretic response seen in Fig. 8A arises. The most critical pieces of information of this analysis are the shape of the response function for the inactivation of Wee1 by CDK1; the shape of the response function for the activation of Cdc25 by CDK1; and the *EC*50 values for both responses. The relevant Wee1 data—both the shape of the response function (sigmoidal, with a Hill coefficient of 3–4) and the *EC*50 (~40 nM)—have been reported [37]. Quantitative studies of Cdc25's responses have more recently been carried out (Trunnell, Kim, and Ferrell, unpublished data). It will certainly be of interest to see whether the Wee1 and Cdc25 responses are sufficient to explain the observed hysteretic response of the cyclin–CDK1 system, or whether some essential element of the system is still missing from our picture of the mitotic trigger.

3.3. Bistability is a recurring theme in cell signaling

Here we have seen two examples of complex biological phenomena—*Xenopus* oocyte maturation and the *Xenopus* embryonic cell cycle—that can be understood in terms of feedback loops, bistability, and saddle–node bifurcations. The idea that positive feedback loops or double-negative feedback loops could be important components of cellular regulatory networks goes back to Delbrück, Novick and Weiner, and Monod and Jacob [17,38,39]. Over the past decade numerous examples of positive feedback loops and bistability have been hypothesized and/or established in eukaryotic systems [40–48]. Arguably the *Xenopus* oocyte and *Xenopus* embryo have led the way in this area [10,14,29,37,49,50], in part because of the unique array of

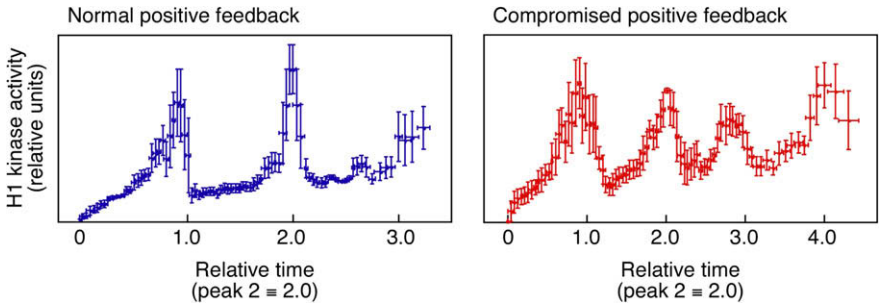

Fig. 7. Short-circuiting the positive feedback in the embryonic cell cycle causes CDK1 oscillations to become less explosive and more damped. Data are from four independent experiments and are pooled, scaled, and expressed as binomially-weighted running averages. Adapted from [29].

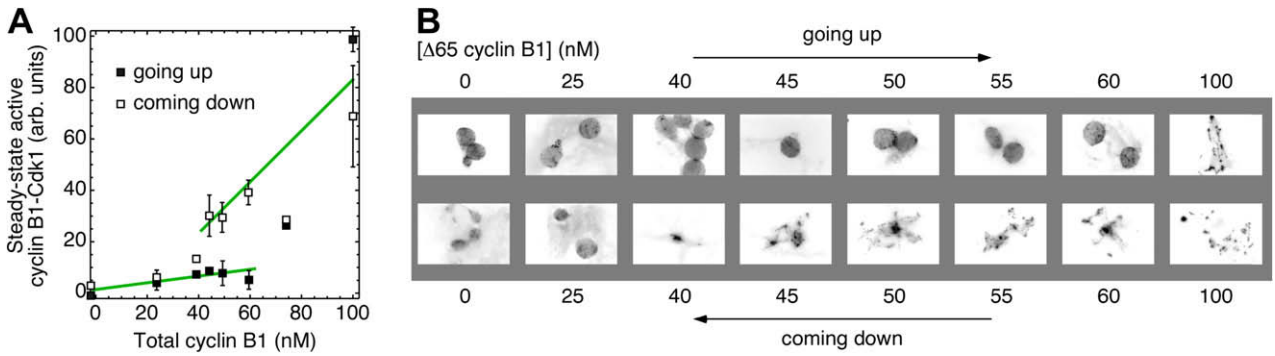

Fig. 8. Hysteresis and bistability in the response of CDK1 to non-degradable cyclin in *Xenopus* egg extracts. (A) CDK1 activity. (B) Morphology of Hoechst-stained sperm chromatin, shown as a negative image. Adapted from [50].

experimental tools available for the biochemical dissection of these systems.

3.4. Bistability in oocyte maturation makes sense

Oocyte maturation is, at its heart, an all-or-none and irreversible process, yet it is built out of components that are graded and reversible in terms of their intrinsic regulation. Both the all-or-none character and the irreversibility of oocyte maturation depend upon positive feedback. The positive feedback loops in the control system together with the sigmoidal response functions present within the feedback loops combine to generate bistability, and the maturation of an oocyte can be understood as a traversal of a saddle–node bifurcation, where the stable steady state representing the immature oocyte ceases to exist, and the oocyte falls up to a stable, self-reinforcing, actively-maintained steady state representing the mature oocyte. Despite many differences in terms of the components and the exact wiring of the control systems, oocyte maturation is related at a fundamental level to the lambda phage lysis–lysogeny switch, to the galactose switch in yeast, and to the process of myogenesis [51].

3.5. Bistability in the embryonic cell cycle is less obviously sensible

The embryonic cell cycle can be thought of as a succession of switches between interphase and mitosis, but, unlike the maturing oocyte, the dividing embryo never rests in either state. At heart the cell cycle is a dynamical process driven by a never-pausing biochemical oscillator. Nevertheless, this oscillator is built upon a switch—the bistable mitotic trigger, which uses a system of interconnected positive and double-negative feedback loops to convert a continuously-variable input (the total concentration of the mitotic cyclins) into a hysteretic response (the activity of cyclin–CDK1). This could help to ensure that the transitions between cell cycle phases are decisive and irreversible, and indeed experimental evidence has now been presented arguing that positive feedback and bistability are critical for the irreversibility of mitotic exit [52].

Bistability also potentially provides the embryonic cell cycle with different performance characteristics than it might have if it were driven by negative feedback alone. For example, with models of negative-feedback-only oscillators, it is usually not possible to tune the period of the oscillations much without markedly changing the oscillator's amplitude; with the combination of a bistable trigger and a negative feedback loop, widely-tunable oscillations become possible [53]. Perhaps this is what allows the embryonic cell cycle to proceed over a wide range of ambient temperatures and cell cycle periods. Bistability may also help coordinate spatially separated regions of a large cell like a *Xenopus* egg: bistability could allow the activity of a regulator like cyclin–CDK1 to spread over millimeter distances much faster than diffusion would. Experiments to test these possibilities are underway.

3.6. Simple models of not so simple systems

It is abundantly clear that biological systems are highly complex. Every eukaryotic cell expresses tens of thousands of genes, and every protein is directly or indirectly connected to every other protein. Despite this complexity, it may be possible to understand how some complex biological processes occur in fairly simple terms. Even though both oocyte maturation and the embryonic cell cycle involve, undoubtedly, dozens of proteins and RNA species (or more), and detailed models would involve dozens of variables and scores of parameters, one can arguably understand the essence of the processes in terms of simple models with one or two time dependent variables and a handful of parameters. The conceptualization of oocyte maturation as the traversal of a saddle–node bifurcation, and the conceptualization of the embryonic cell cycle as an oscillator built upon a bistable switch with two saddle–node

bifurcations, provide examples of how some very "biological" behaviors can be understood in simple terms. The tools for achieving this type of understanding include quantitative experimental approaches, numerical modeling, the analytical methods of nonlinear dynamics, and a willingness to pare complex systems down to the simplest representation consistent with experiment. The necessary input information includes, of course, the wiring diagrams for the system, but also the qualitative shapes of the response functions as well as some quantitative measures the how the system responds (abundances, rate constants, *EC*50's, and, if dynamical rather than steady-state properties are being considered, time lags).

References

[1] Murray, A.W. (1991) Cell cycle extracts. Meth. Cell Biol. 36, 581–605.
[2] Bayaa, M., Booth, R.A., Sheng, Y. and Liu, X.J. (2000) The classical progesterone receptor mediates Xenopus oocyte maturation through a nongenomic mechanism. Proc. Natl. Acad. Sci. USA 97, 12607–12612.
[3] Tian, J., Kim, S., Heilig, E. and Ruderman, J.V. (2000) Identification of XPR-1, a progesterone receptor required for Xenopus oocyte activation. Proc. Natl. Acad. Sci. USA 97, 14358–14363.
[4] Boonyaratanakornkit, V., Scott, M.P., Ribon, V., Sherman, L., Anderson, S.M., Maller, J.L., Miller, W.T. and Edwards, D.P. (2001) Progesterone receptor contains a proline-rich motif that directly interacts with SH3 domains and activates c-Src family tyrosine kinases. Mol. Cell 8, 269–280.
[5] Bagowski, C.P., Myers, J.W. and Ferrell Jr., J.E. (2001) The classical progesterone receptor associates with p42 MAPK and is involved in phosphatidylinositol 3-kinase signaling in Xenopus oocytes. J. Biol. Chem. 276, 37708–37714.
[6] Josefsberg Ben-Yehoshua, L., Lewellyn, A.L., Thomas, P. and Maller, J.L. (2007) The role of Xenopus membrane progesterone receptor beta in mediating the effect of progesterone on oocyte maturation. Mol. Endocrinol. 21, 664–673.
[7] Lutz, L.B., Cole, L.M., Gupta, M.K., Kwist, K.W., Auchus, R.J. and Hammes, S.R. (2001) Evidence that androgens are the primary steroids produced by Xenopus laevis ovaries and may signal through the classical androgen receptor to promote oocyte maturation. Proc. Natl. Acad. Sci. USA 98, 13728–13733.
[8] Ferrell Jr., J.E. (1999) Xenopus oocyte maturation: new lessons from a good egg. Bioessays 21, 833–842.
[9] Lew, J. (2003) MAP kinases and CDKs: kinetic basis for catalytic activation. Biochemistry 42, 849–856.
[10] Ferrell Jr., J.E. and Machleder, E.M. (1998) The biochemical basis of an all-or-none cell fate switch in Xenopus oocytes. Science 280, 895–898.
[11] Gotoh, Y., Masuyama, N., Dell, K., Shirakabe, K. and Nishida, E. (1995) Initiation of Xenopus oocyte maturation by activation of the mitogen-activated protein kinase cascade. J. Biol. Chem. 270, 25898–25904.
[12] Matten, W.T., Copeland, T.D., Ahn, N.G. and Vande Woude, G.F. (1996) Positive feedback between MAP kinase and Mos during Xenopus oocyte maturation. Dev. Biol. 179, 485–492.
[13] Roy, L.M., Haccard, O., Izumi, T., Lattes, B.G., Lewellyn, A.L. and Maller, J.L. (1996) Mos proto-oncogene function during oocyte maturation in Xenopus. Oncogene 12, 2203–2211.
[14] Xiong, W. and Ferrell Jr., J.E. (2003) A positive feedback-based bistable "memory-module" that governs a cell fate decision. Nature (London) 426, 460–465.
[15] Sohaskey, M.L. and Ferrell Jr., J.E. (1999) Distinct, constitutively active MAPK phosphatases function in Xenopus oocytes: implications for p42 MAPK regulation in vivo. Mol. Biol. Cell 10, 3729–3743.
[16] Huang, C.-Y.F. and Ferrell Jr., J.E. (1996) Ultrasensitivity in the mitogen-activated protein kinase cascade. PNAS 93, 10078–10083.
[17] Monod, J. and Jacob, F. (1961) General conclusions: teleonomic mechanisms in cellular metabolism, growth, and differentiation. Cold Spring Harbor Symp. Quant. Biol. 26, 389–401.
[18] Becskei, A., Seraphin, B. and Serrano, L. (2001) Positive feedback in eukaryotic gene networks: cell differentiation by graded to binary response conversion. EMBO J. 20, 2528–2535.
[19] Gardner, T.S., Cantor, C.R. and Collins, J.J. (2000) Construction of a genetic toggle switch in *Escherichia coli*. Nature 403, 339–342.
[20] Morgan, D.O. (1997) Cyclin-dependent kinases: engines, clocks, and microprocessors. Annu. Rev. Cell Dev. Biol. 13, 261–291.
[21] Morgan, D.O. (2007) The Cell Cycle: Principles of Control, New Science Press Ltd., London, UK.
[22] Murray, A.W. and Kirschner, M.W. (1989) Dominoes and clocks: the union of two views of the cell cycle. Science 246, 614–621.
[23] Loog, M. and Morgan, D.O. (2005) Cyclin specificity in the phosphorylation of cyclin-dependent kinase substrates. Nature 434, 104–108.
[24] Ubersax, J.A., Woodbury, E.L., Quang, P.N., Paraz, M., Blethrow, J.D., Shah, K., Shokat, K.M. and Morgan, D.O. (2003) Targets of the cyclin-dependent kinase Cdk1. Nature 425, 859–864.
[25] Murray, A.W., Solomon, M.J. and Kirschner, M.W. (1989) The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. Nature 339, 280–286.
[26] Goodwin, B.C. (1965) Oscillatory behavior in enzymatic control processes in: Advances in Enzyme Regulation (Weber, G., Ed.), pp. 425–438, Pergamon Press, Oxford, UK.
[27] Goldbeter, A. (1991) A minimal cascade model for the mitotic oscillator involving cyclin and cdc2 kinase. Proc. Natl. Acad. Sci. USA 88, 9107–9111.
[28] Elowitz, M.B. and Leibler, S. (2000) A synthetic oscillatory network of transcriptional regulators. Nature 403, 335–338.
[29] Pomerening, J.R., Kim, S.Y. and Ferrell Jr., J.E. (2005) Systems-level dissection of the cell-cycle oscillator: bypassing positive feedback produces damped oscillations. Cell 122, 565–578.
[30] Solomon, M.J., Gautier, J., Lee, T. and Kirschner, M.W. (1991) Control of p34cdc2 activation. Cold Spring Harb. Symp. Quant. Biol. 56, 427–435.
[31] Solomon, M.J., Glotzer, M., Lee, T.H., Philippe, M. and Kirschner, M.W. (1990) Cyclin activation of $p34^{cdc2}$. Cell 63, 1013–1024.
[32] Novak, B. and Tyson, J.J. (1993) Numerical analysis of a comprehensive model of M-phase control in Xenopus oocyte extracts and intact embryos. J. Cell Sci. 106, 1153–1168.
[33] Thron, C.D. (1996) A model for a bistable biochemical trigger of mitosis. Biophys. Chem. 57, 239–251.
[34] Kumagai, A. and Dunphy, W.G. (1992) Regulation of the cdc25 protein during the cell cycle in Xenopus extracts. Cell 70, 139–151.
[35] Hoffmann, I., Clarke, P.R., Marcote, M.J., Karsenti, E. and Draetta, G. (1993) Phosphorylation and activation of human cdc25-C by cdc2–cyclin B and its involvement in the self-amplification of MPF at mitosis. EMBO J. 12, 53–63.
[36] Mueller, P.R., Coleman, T.R. and Dunphy, W.G. (1995) Cell cycle regulation of a Xenopus Wee1-like kinase. Mol. Biol. Cell 6, 119–134.
[37] Kim, S.Y. and Ferrell Jr., J.E. (2007) Substrate competition as a source of ultrasensitivity in the inactivation of Wee1. Cell 128, 1133–1145.
[38] Delbruck, M. (1949) DiscussionUnités Biologiques douées de Continuité Génétique, pp. 33–35, Colloques Internationaux du CNRS, Paris.
[39] Novick, A. and Wiener, M. (1957) Enzyme induction as an all-or-none phenomenon. Proc. Natl. Acad. Sci. USA 43, 553–566.
[40] Lisman, J.E. (1985) A mechanism for memory storage insensitive to molecular turnover: a bistable autophosphorylating kinase. Proc. Natl. Acad. Sci. USA 82, 3055–3057.
[41] Bagowski, C.P. and Ferrell, J.E. (2001) Bistability in the JNK cascade. Curr. Biol. 11, 1176–1182.
[42] Cross, F.R., Archambault, V., Miller, M. and Klovstad, M. (2002) Testing a mathematical model of the yeast cell cycle. Mol. Biol. Cell 13, 52–70.
[43] Mullasseril, P., Dosemeci, A., Lisman, J.E. and Griffith, L.C. (2007) A structural mechanism for maintaining the 'on-state' of the CaMKII memory switch in the post-synaptic density. J. Neurochem. 103, 357–364.
[44] Skotheim, J.M., Di Talia, S., Siggia, E.D. and Cross, F.R. (2008) Positive feedback of G1 cyclins ensures coherent cell cycle entry. Nature 454, 291–296.
[45] Di Talia, S., Skotheim, J.M., Bean, J.M., Siggia, E.D. and Cross, F.R. (2007) The effects of molecular noise and size control on variability in the budding yeast cell cycle. Nature 448, 947–951.
[46] Holt, L.J., Krutchinsky, A.N. and Morgan, D.O. (2008) Positive feedback sharpens the anaphase switch. Nature 454, 353–357.
[47] Chakraborty, A.K., Das, J., Zikherman, J., Yang, M., Govern, C.C., Ho, M., Weiss, A. and Roose, J. (2009) Molecular origin and functional consequences of digital signaling and hysteresis during Ras activation in lymphocytes. Sci Signal 2 (pt2).
[48] Das, J., Ho, M., Zikherman, J., Govern, C., Yang, M., Weiss, A., Chakraborty, A.K. and Roose, J.P. (2009) Digital signaling and hysteresis characterize ras activation in lymphoid cells. Cell 136, 337–351.
[49] Sha, W., Moore, J., Chen, K., Lassaletta, A.D., Yi, C.S., Tyson, J.J. and Sible, J.C. (2003) Hysteresis drives cell-cycle transitions in *Xenopus laevis* egg extracts. Proc. Natl. Acad. Sci. USA 100, 975–980.
[50] Pomerening, J.R., Sontag, E.D. and Ferrell Jr., J.E. (2003) Building a cell cycle oscillator: hysteresis and bistability in the activation of Cdc2. Nat. Cell Biol. 5, 346–351.
[51] Brandman, O., Ferrell Jr., J.E., Li, R. and Meyer, T. (2005) Interlinked fast and slow positive feedback loops drive reliable cell decisions. Science 310, 496–498.
[52] Lopez-Aviles, S., Kapuy, O., Novak, B. and Uhlmann, F. (2009) Irreversibility of mitotic exit is the consequence of systems-level feedback. Nature 459, 592–595.
[53] Tsai, T.Y., Choi, Y.S., Ma, W., Pomerening, J.R., Tang, C. and Ferrell Jr., J.E. (2008) Robust, tunable biological oscillations from interlinked positive and negative feedback loops. Science 321, 126–129.