Modelling the controls of the eukaryotic cell cycle

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Abstract

The eukaryotic cell-division cycle is regulated by three modules that control G_1/S, G_2/M and meta/anaphase transitions. By using mathematical modelling, we show the dynamic characteristics of these individual modules and we also assemble them together into a comprehensive model of the eukaryotic cell-division cycle. With this comprehensive model, we also discuss the mechanisms by which different checkpoint pathways stabilize different cell-cycle states and inhibit the transitions that drive cell-cycle progression.

Physiology of the cell cycle

During its division cycle, a cell must replicate all of its components and divide them into two nearly identical daughter cells [1]. Since DNA stores the genetic information, it must be accurately replicated (during the S phase of the cycle). DNA replication results in two identical sister-chromatids, which must be precisely segregated. Segregation of sister-chromatids happens during mitosis (M phase). There are characteristic gaps between these two cell-cycle events: G_1 phase between mitosis and S phase, and G_2 phase between S and M phases.

There are three irreversible transitions during the normal mitotic cycle [2]. (i) Start, when the cell commits itself to cell division instead of choosing an alternative developmental process (e.g. mating or sporulation). (ii) G_2/M transition, when the cell commits itself to enter into mitosis. (iii) Finish, when the cell exits from mitosis (anaphase, telophase, cell division).

Cells are driven through the cell cycle by an underlying molecular engine consisting of protein molecules that interact with each other in a complicated way. The cell-cycle engine must satisfy at least three requirements in order to drive a successful cell cycle [2]. (i) It must trigger the S phase first and mitosis later, so that the S and M phases always alternate during the normal mitotic cell cycle. (ii) The engine must be subject to checkpoint controls: if one cell-cycle event cannot happen for some reason, then further progress through the cycle must be blocked [3]. (iii) Balanced growth and division: a cell must double its cytoplasmic mass between two successive divisions. This requirement is satisfied if the cell-cycle engine is sensitive to the cytoplasmic mass per DNA ratio, because in this case the engine will trigger cell-cycle events with the periodicity of the mass-doubling time.

Key words: bifurcation, cell cycle, checkpoint, mathematical model.

Abbreviations used: Cdk, cyclin-dependent kinase; APC, anaphase-promoting complex.

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The molecular network of the cell-cycle engine

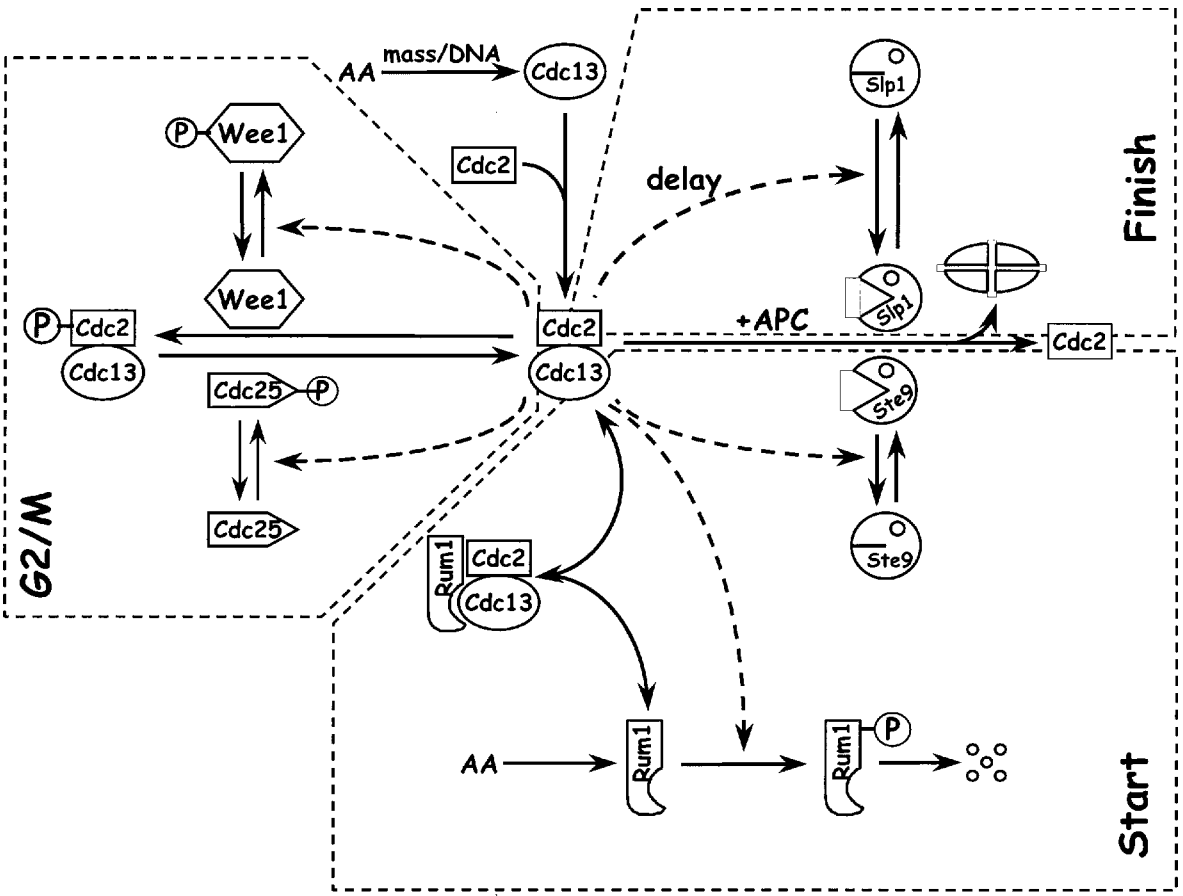
The most important components of the cell-cycle engine are a special family of protein kinases, called Cdks (cyclin-dependent kinases). Cdks are heterodimers, consisting of a catalytic kinase subunit and a regulatory cyclin subunit, which is essential for the protein-kinase activity [4].

In higher eukaryotes there are many Cdk–cyclin complexes, which trigger different cell-cycle events. In lower eukaryotes, like fission yeast, a single Cdk–cyclin complex can drive the whole cell cycle [5]. This complex is called Cdc2–Cdc13 in fission yeast: Cdc2 is the Cdk subunit and Cdc13 is the major B-type cyclin in fission yeast cells [6]. Cdc2 is present at a constant level throughout the cell cycle, and it is in excess over Cdc13. Cdc13 is continuously synthesized and it combines with Cdc2 to form an active Cdk–cyclin complex. The Cdc2–Cdc13 complex formed is not necessarily active, because cells have at least three different mechanisms to down-regulate its activity (Figure 1). (i) Degradation of Cdc13 subunit, which is mediated by the APC (anaphase-promoting complex), which requires Slp1 protein to recognize Cdc13 [7]. Degradation of Cdc13 is primarily responsible for the Finish transition. (ii) Inhibitory phosphorylation, which is mediated by Wee1 kinase, whose action is reversed by Cdc25 activatory phosphatase. Inhibitory phosphorylation is used for the regulation of the G_2/M transition. (iii) Binding of a stoichiometric inhibitor (Rum1) to the Cdc2–Cdc13 complex. Rum1 is continuously synthesized; however, if it becomes phosphorylated, it is rapidly destroyed [8]. The Rum1 Cdk-inhibitor stabilizes the G_1 state and is destroyed at the Start transition [9,10]. G_1 is also stabilized by cyclin proteolysis [11,12], mediated by Ste9/APC (like Slp1, Ste9 is used by APC to recognize Cdc13).

All the proteins influencing Cdc2–Cdc13 activity in fact are regulated by Cdc2–Cdc13 activity themselves, thereby creating feedback loops in the network [13]. There are three types of feedback loop in the cell-cycle engine [14].

The first is double negative feedback: Rum1, Wee1 and Ste9 have negative effects on Cdc2–Cdc13 kinase,

Figure 1 | Cell-cycle regulation in fission yeast
AA, amino acids.



reducing its activity by different mechanisms. On the other hand, Cdc2–Cdc13 has negative effects on these molecules by phosphorylating them: phosphorylation of Wee1 and Ste9 reduces their activities [11,12,15], while phosphorylation of Rum1 promotes its degradation [8]. As a consequence these molecules and Cdc2–Cdc13 have a mutual antagonistic relationship (double negative loop). Double negative feedback has many characteristics common with positive feedback.

Second is positive feedback: Cdc25 antagonizes Wee1 action, thereby activating Cdc2-Cdc13. In return, Cdc2–Cdc13 activates Cdc25 by phosphorylation, thereby creating a positive feedback loop in the mechanism [16].

Third is the time-delayed negative feedback loop: Slp1 has a negative effect on Cdc2–Cdc13 kinase by promoting the degradation of the Cdc13 component. Because Cdc2–Cdc13 activates Slp1/APC, although possibly not directly, a negative feedback loop is established.

The network shown on Figure 1 is a consensus picture about Cdc2–Cdc13 regulation in fission yeast, which is based on a great amount of experimental data. Since the network is complicated by feedback controls, its operation cannot be understood simply by verbal arguments. To understand how the molecular pieces determine the behaviour of the whole control system requires an appropriate and precise scientific tool, namely mathematical modelling.

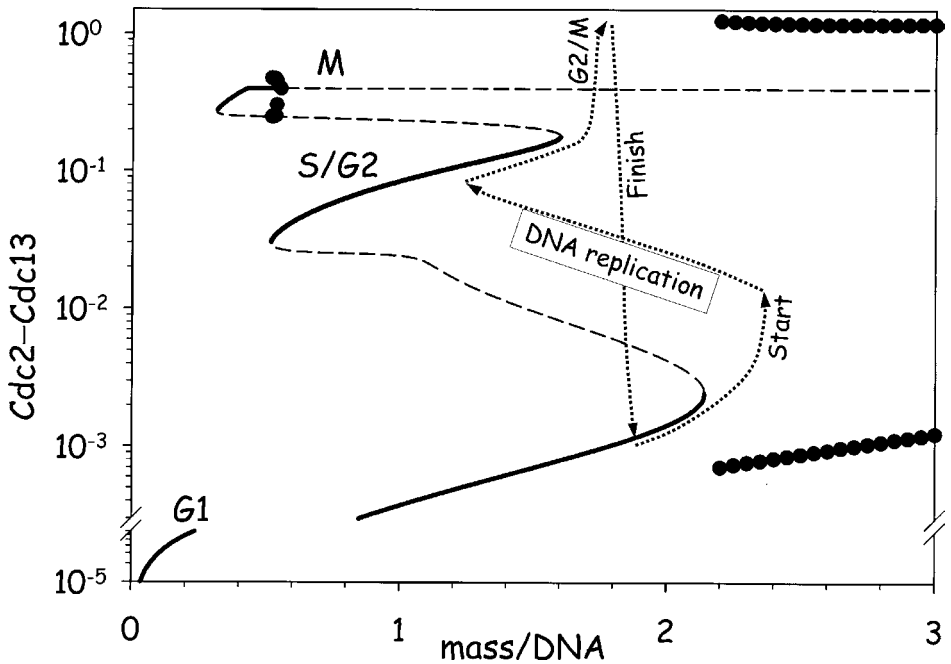
Mathematical modelling of the cell-cycle engine

By writing differential equations for the time rate of change of all the components in this network, the network can be transformed into a mathematical model, which carries all the information shown on the wiring diagram in a computer-readable form [17]. These equations can be used to find out all the possible characteristic states of the control system: steady states and periodic solutions, which can be either stable or unstable.

Which of the 'recurrent' states is manifested, as well as their stability, is determined by the numerical values of parameters in the model. The dynamics of the cell-cycle control network are highly influenced by the mass/DNA ratio of the cell, which can be thought of as a slow-changing parameter during the cycle. In order to characterize the influence of the mass/DNA ratio on the cell-cycle control network, the state of the control system needs to be characterized as a function of mass/DNA [18]. The steady state and the periodic solutions can be characterized by any molecular component of the

Figure 2 | Bifurcation diagram for wild-type fission yeast cell cycle

Solid lines, stable steady states; dashed lines, unstable steady states; filled circles, minima and maximum of oscillation. The 'orbit' of cycling cells is indicated by dotted arrows.



network, but the most obvious choice is the concentration of Cdc2–Cdc13, because it is a master regulator of the cell cycle.

On the plot of Cdc2–Cdc13 activity (in the nucleus) as a function of mass/DNA (see Figure 2), we find three different stable steady states (solid lines) with characteristically different Cdk activity. The steady state with very low Cdc2–Cdc13 activity corresponds to the G_1 phase of the cycle (Rum1 level is high and Ste9 is active). In the steady state with intermediate Cdc2–Cdc13 activity, Wee1 is active and Cdc25 is inactive, and we associate these states with the S/G_2 phase of the cell cycle (in the model there is no difference between the S and G_2 phases except that DNA replication is ongoing or not). Finally, the steady state with high Cdc2–Cdc13 activity corresponds to the mitotic state. G_1, S/G_2 and M states are separated by unstable steady states (so called saddle points, indicated by dashed lines), which are not directly observable experimentally, but they play important roles. The high-Cdk-activity mitotic state is stable for small mass/DNA values, but it soon becomes unstable (Figure 2, dashed lines). For larger values of mass/DNA (>2.2), these unstable steady states are surrounded by stable limit cycle oscillations (on Figure 2 the minima and maxima are indicated by filled circles), which are driven by the negative feedback loop of the Finish module.

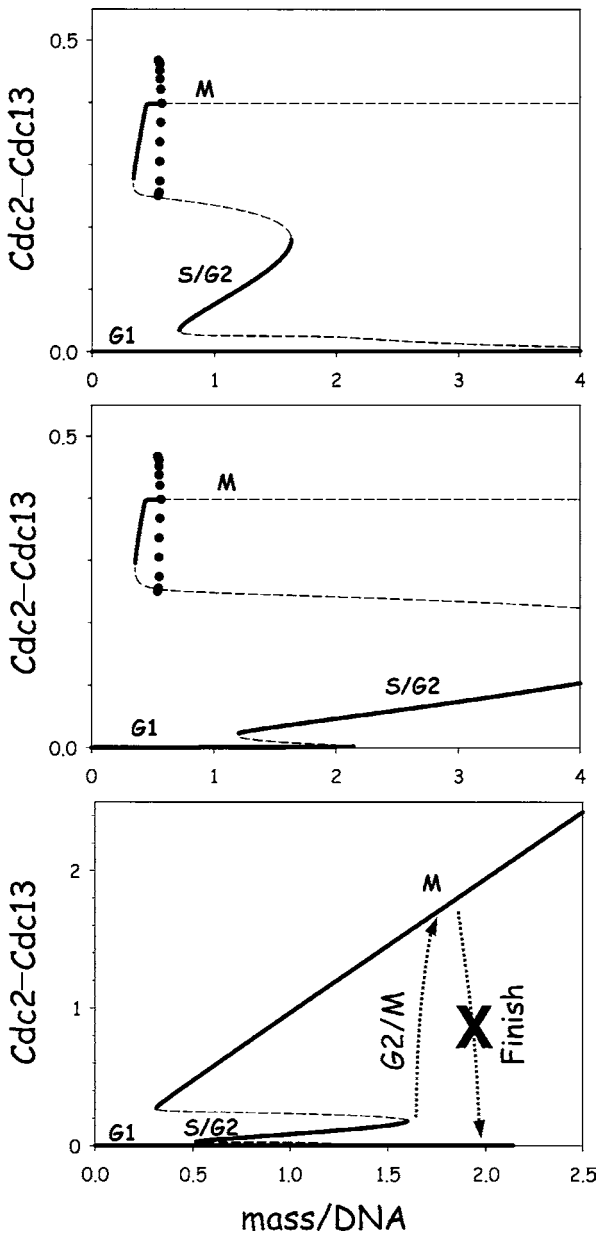
Cycling cells are moving along this diagram in a characteristic way. G_1 cells find themselves close to the stable G_1 state. As they are growing their mass/DNA ratio reaches a critical value where the G_1 state disappears and they find themselves in the oscillatory regime. As a consequence the Cdc2–Cdc13 activity starts to increase (Start) and triggers DNA replication. DNA duplication during S phase causes a halving of the mass/DNA ratio which removes the cell from the oscillatory regime. Actually the cell ends up in a stable G_2 state. Since the mass/DNA ratio increases further the cell reaches a critical point where the S/G_2 state disappears. Now the Cdc2–Cdc13 activity rises abruptly past the unstable mitotic states, which marks the G_2/M transition. High Cdc2–Cdc13 activity brings about all the events of mitosis and finally activates degradation of Cdc13. The drop in Cdc2–Cdc13 activity drives the cell out of mitosis (Finish transition) back to the stable G_1 state where we started this description.

Cell-cycle checkpoints

If a cell-cycle event cannot be completed, then a checkpoint mechanism blocks (or delays) further cell-cycle progression [2,3]. Activation of a checkpoint mechanism has a characteristic effect on the bifurcation diagram. There are three characteristic bifurcation points on the diagram [18]. (i) Where the stable G_1 state disappears (i.e. where it coalesces with the unstable saddle point) determines when cells can undergo Start. (ii) The mass/DNA value where the stable S/G_2 state disappears, determines when the G_2/M transition can take place. (iii) Cells can come out of mitosis (Finish transition) at mass/DNA values where the mitotic state is unstable.

When a checkpoint gets activated then a signal transduction pathway detects the problem and transmits an inhibitory signal to the cell-cycle engine [3]. The signal generally up-regulates one of the negative regulators of Cdc2–Cdc13,

Figure 3 | Bifurcation diagram for checkpoint control
Top, G_1 checkpoint; middle, G_2 checkpoint; bottom, metaphase checkpoint. Symbols are the same as on Figure 2.



depending on which checkpoint pathway was activated [13]. As a consequence of checkpoint activation, the characteristic bifurcation point moves to a higher mass/DNA ratio [18], thereby blocking or delaying the next cell-cycle transition (see Figure 3). (i) If yeast cells are exposed to mating pheromone of the opposite mating type, then the G_1 state gets extended, thereby lengthening G_1 phase. (ii) If DNA replication cannot be completed, then the S/G_2 state extends to a much higher mass/DNA ratio, thereby blocking the G_2/M transition. (iii) If spindle assembly is blocked, then the point where the M state becomes unstable moves to a much higher mass/DNA value. As a consequence, mitosis becomes a stable state, and cells entering into M phase will be stuck there.

Conclusions

Our aim is to make connections between molecular control systems and cell physiology. To do so requires that we look at the problem from three different points of view: the molecular network of the control mechanism, its transformation into differential equations, and its analysis by dynamical system theory. These three points of view complement each other, and together they give us an in-depth understanding of the dynamics of the network and how it really plays out in the physiology of the cell. The molecular network is the natural view of molecular geneticists. Ideas from the theory of dynamical systems, like bistability and hysteresis, are the natural language of modellers. The differential equations provide a machine-readable form of these ideas, allowing both experimentalist and theoretician to explore the relations between their hypothetical molecular mechanisms and the actual behaviour of living cells.

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