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Review

System-level feedbacks control cell cycle progression

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ABSTRACT

Repetitive cell cycles, which are essential to the perpetuation of life, are orchestrated by an underlying biochemical reaction network centered around cyclin-dependent protein kinases (Cdks) and their regulatory subunits (cyclins). Oscillations of Cdk1/CycB activity between low and high levels during the cycle trigger DNA replication and mitosis in the correct order. Based on computational modeling, we proposed that the low and the high kinase activity states are alternative stable steady states of a bistable Cdk-control system. Bistability is a consequence of system-level feedback (positive and double-negative feedback signals) in the underlying control system. We have also argued that bistability underlies irreversible transitions between low and high Cdk activity states and thereby ensures directionality of cell cycle progression.

1. Introduction

All cells arise by the division of preexisting cells. Successful cell division requires that the cell first replicate its DNA (during the period called S phase), and then partition the replicated chromosomes evenly to the two incipient progeny cells in mitosis (M phase). During cell proliferation, chromosome replication and segregation should happen once and only once between two successive divisions and in the correct order: S phase followed by M phase. The timing and ordering of chromosome replication, segregation and cell division (in eukaryotes) is controlled by cyclin-dependent protein kinases (Cdks) and their counter-acting phosphatases. Cdks in complex with activatory subunits (called cyclins) phosphorylate their target proteins on specific amino acid residues, while phosphatases reverse these modifications [1].

These target proteins are responsible for executing particular cell cycle events. Hence the proper ordering of these events depends on the timely activation (or possibly inactivation) of Cdk-target proteins. Because Cdk-dependent phosphorylation events may either activate or inhibit target proteins, Cdks can both activate and inhibit particular cell cycle events, and this dual control is crucial for proper progression through the cell cycle [2].

The Cdk/cyclin complexes that initiate S phase and mitosis are conveniently called S phase promoting factor (SPF) and M phase promoting factor (MPF), respectively [3]. Higher eukaryotes have many different Cdks (Cdk1, Cdk2, etc.) and multiple cyclins (CycA, CycB, etc.); however, only Cdk1 is essential [4]. In higher eukaryotes the Cdk subunits of SPF and MPF are Cdk1 and Cdk2, respectively; while lower eukaryotes employ Cdk1 in both SPF and MPF. The cyclin partners in SPF and MPF are usually different: CycA and CycB in higher eukaryotes, and two different CycBs in lower eukaryotes.

SPF activity rises at the G1/S transition and triggers DNA replication, while MPF activity peaks later and brings about M phase. Since both SPF and MPF block further replication of already replicated chromosomes, re-replication of the genome is impossible as long as these activities are present. At the end of mitosis both SPF and MPF activities disappear because their cyclin subunits are degraded [5]. During G1 phase of the following cell cycle, when both SPF and MPF activities are low [6], origins of replication can be relicensed for another round of DNA synthesis [7,8].

Lumping together SPF and MPF activities, we can define two fundamental states of the cell cycle: G1, when Cdk activity is low and chromosomes are unreplicated, and S + G2 + M, when Cdk activity is high and chromosomes are being replicated and segregated. Two fundamental transitions define the boundaries between

Abbreviations: Cdk, cyclin-dependent-kinase; APC, anaphase promoting complex; CKI, cyclin-dependent kinase inhibitor; SPF, S phase promoting factor; MPF, M phase promoting factor

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these two states: the G1/S transition (sometimes called 'Start') and the M/G1 transition (often called 'Exit from mitosis'). The two fundamental cell cycle states must be stable, and the transitions between them must be irreversible. An accidental transition from one state to the other can compromise the order of cell cycle events. If, for example, the cell loses SPF and MPF activities before it has completed mitosis, then the next activation of SPF will trigger another round of S phase and increase the ploidy of the cell [9]. Hence, the underlying mechanisms that provide stability to the low- and high-Cdk states and irreversibility to the transitions between these states are crucial for unidirectional progression through the cell cycle.

The cyclin components of both SPF and MPF are abruptly degraded at the end of mitosis [10], and this observation provided a simple, intuitive explanation for the irreversibility of mitotic exit: since protein degradation is a thermodynamically irreversible process, a dividing cell cannot revert to M phase [11]. Tim Hunt's discovery of cyclin degradation became a paradigm for understanding cell cycle progression, as many other cell cycle regulators were found to be abruptly degraded in specific phases of the cycle. Therefore, it is commonplace now to explain all cell cycle transitions by the irreversibility of protein degradation [11].

But there is a flaw in this simple, appealing view: in a living cell, protein degradation is counteracted by protein synthesis. Consequently, although protein degradation is thermodynamically irreversible, the concentration of a cellular protein may rise or fall reversibly with the shifting kinetic balance between protein synthesis and degradation [12]. As an alternative to the paradigm that 'protein degradation provides directionality to the cell cycle', Novak and Tyson have been arguing for many years that the irreversibility of cell cycle transitions is an emergent property of feedback circuits in the molecular mechanism of Cdk control [13–15].

2. Systems view of cell cycle control

Cdk subunits are usually present in a cell at high concentrations, and the cell modulates the availability of cyclin subunits as one means of controlling Cdk activities. Cyclin levels are modulated by controls on both cyclin synthesis and degradation. Rates of cyclin synthesis are regulated by transcription factors. Cyclin degradation is initiated by ubiquitination of cyclin subunits by the Anaphase Promoting Complex (APC; also called the 'Cyclosome') [16]. The rate of cyclin ubiquitination can be modulated by phosphorylation and dephosphorylation of the APC and its associated proteins, Cdc20 and Cdh1.

Besides the regulation of cyclin levels, eukaryotes control SPF and MPF kinase activities by other mechanisms. For example, most cells in G1 phase have stoichiometric inhibitors (CKIs) that bind to and inhibit Cdk/cyclin complexes [17–19]. In addition, reversible phosphorylation of the Cdk1 subunit may be used to inhibit MPF activity, especially in cells with a long G2 phase [20].

With the exception of cyclin synthesis, all the processes mentioned above have a negative effect on SPF and MPF activities. In addition, all the proteins regulating SPF and MPF activities are among the target proteins of SPF and MPF as well. Therefore, feedback loops are created in the cell cycle control system, and these loops entail dynamic properties not evident in the individual components.

In most cases, SPF and MPF down-regulate their inhibitory proteins, creating a double-negative ('antagonistic') feedback loop. When they are activating their positive regulators, a positive feedback loop is established. Importantly, both of these network motifs can potentially create a bistable system with alternative stable steady states [21]. Based on physiological arguments, we have proposed that the low- and high-activity states of Cdk observed during the cell cycle are two alternative steady states of the underlying Cdk-control network (see [22] for review).

Switching between these two stable states, which must occur at the G1/S and M/G1 transitions, is promoted by 'helper proteins' (see Fig. 1). Helper proteins are not part of these feedback loops, but they can push the bistable switch in one direction or the other. The helper proteins for the G1/S transition are starter kinases (SK, usually Cdk/cyclin complexes other than SPF and MPF) that are resistant to the inhibitors of SPF and MPF and are not dependent on the activators of SPF and MPF. By inhibiting the inhibitors of SPF and MPF (or activating their activators), the starter kinases destabilize the G1 state of the cycle and promote entry into S phase. 'Exit proteins' (EP) work in the opposite way to destabilize the high Cdk activity state and promote exit from mitosis.

Since Start and Exit flip the switch in opposite directions, the helper proteins for one transition are inhibitory for the other. Therefore, the helper proteins must be present only transiently during the Start and Exit transitions. This transiency is achieved by negative feedback loops in the control system. The starter kinases are down-regulated by high SPF and MPF activities after the G1/S transition takes place. In contrast, the activities of exit proteins are dependent on high SPF and/or MPF activity, and, hence, they turn off after mitotic exit. As a consequence of these negative feedback loops, after each transition a cycling cell returns to a neutral state of the control system (no starter kinases and no exit proteins). In the neutral state, the control system is bistable. It locks into the high Cdk activity state after the G1/S transition and into the low activity state after the M/G1 transition. Consequently, this bistable switch with two negative feedback loops drives the cell cycle control system (Fig. 1) in a clockwise direction like a ratchet. This ratcheting effect (which engineers call 'hysteresis') ensures strict alternation between the low and high Cdk activity states, thereby guaranteeing the correct order of cell cycle events.

Progression around this hysteresis loop is also controlled by checkpoint mechanisms. These surveillance mechanisms keep the control system in one of the stable states until certain conditions

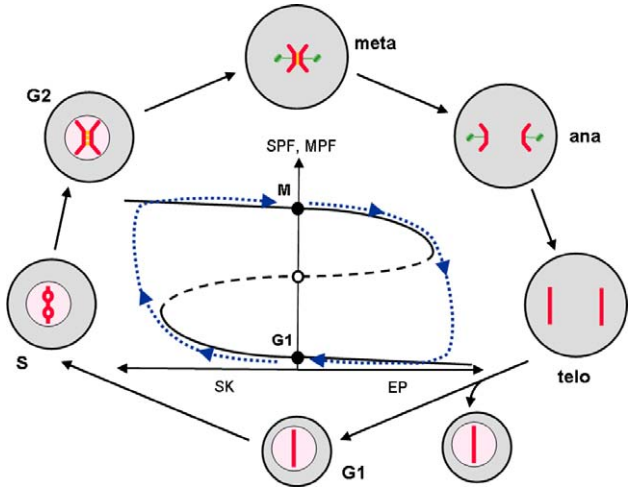

Fig. 1. Alternative, stable states in the eukaryotic cell cycle. SPF and MPF are involved in positive and double-negative feedback loops with their regulators. These feedback loops can persist in either one of two stable states: G1 with low activities of SPF and MPF, and S/G2/M with high activities. These two stable states are represented by continuous lines on the diagram, and they are separated by a locus of unstable steady states indicated by the dashed line. Starter kinases (SK) destabilize the G1 state by inhibiting the antagonists of SPF and MPF (and possibly by activating their activators). Exit proteins (EP) have the opposite effect on the regulators of SPF and MPF regulators, thereby promoting return to the G1 state. Eukaryotic cells move clockwise around this bistable switch, as indicated by the blue trajectory (often referred to as a 'hysteresis loop') and the typical cytological stages of the cell cycle surrounding the diagram.