Cell Cycle Vignettes

Irreversible Transitions in the Cell Cycle

John J. Tyson & Bela Novak
Department of Biological Sciences
Virginia Polytechnic Institute & State University
Blacksburg, Virginia USA
&
Centre for Integrative Systems Biology
Oxford University, Oxford UK

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The cell cycle is the sequence of events whereby a cell replicates its chromosomes and partitions the identical sister chromatids into two separate nuclear compartments (Morgan, 2007). In eukaryotic cells, the phases of DNA synthesis (S phase) and mitosis (M phase) are temporally distinct and separated by gap phases (G1—unreplicated chromosomes, and G2—replicated chromosomes). To maintain the proper ploidy of a cell lineage, generation after generation, it is essential that S and M phases are strictly alternating. This alternation is enforced by the irreversibility of three crucial transitions in the cell cycle: the G1/S, G2/M and M/G1 transitions (Novak et al, 2007). By 'irreversibility' we understand that, once cells have committed to a new round of DNA replication (at the G1/S transition), they do not typically slip back into G1 phase and do a second round of DNA replication. Similarly, once cells have committed to mitosis (at the G2/M transition), they do not typically slip back into G2 phase and try for a second mitotic division.

Like most 'rules' in biology, this one has important exceptions (Morgan, 2007). Some cells carry out repeated rounds of DNA replication without intervening mitoses, becoming polyploid. During meiosis, a germ line cell undergoes two meiotic divisions without an intervening S phase. But these exceptions only reinforce the centrality of the general rule of somatic cell cycles, namely, irreversible progression through the cell cycle phases (G1, S, G2, M) in strict order.

The irreversibility of the three transitions is intimately connected with cell cycle checkpoints, which halt further progression through the cell cycle whenever serious problems are detected (Murray, 1992). For example, DNA damage incurred during G1 phase will block the G1/S transition until the damage is repaired. Failure to fully replicate and ligate DNA molecules during S phase will block the G2/M transition. Incomplete alignment of replicated chromosomes on the mitotic spindle will block the M/G1 transition. When a checkpoint is lifted, the cell proceeds irreversibly to the next phase of the cell cycle.

These irreversible transitions and checkpoints are controlled by complex molecular regulatory networks with both positive and negative feedback. Positive feedback creates a bistable switch, and negative feedback allows the switch to be flipped from one stable state to another (Tyson & Novak, 2008). These systems-level properties of the regulatory network are crucial to irreversible progression through the cell cycle, and when they are disturbed by mutation, drugs or disease, then cells make mistakes in DNA replication and partitioning, often with fatal consequences for the cell or for the multicellular organism harboring the rogue cells.

Physiology and molecular biology

Progression through the cell cycle is governed by a set of cyclin-dependent kinases (CDKs) that initiate DNA replication and mitosis and that inhibit the transition from metaphase to anaphase-telophase-cytokinesis ('exit from mitosis'). G1 phase cells are uncommitted to the cell cycle because they lack the necessary CDK activities. They are devoid of S- and M-phase cyclins because the transcription factors that would drive production of these cyclins are inactive, and the ubiquitin-ligating enzymes (ULE) that label these cyclins for proteolysis are active. In addition, G1 phase cells contain generous amounts of CDK inhibitors (CKIs) that bind to and inhibit CDK-cyclin dimers.

At the G1/S transition (called 'Start' in budding yeast and the 'Restriction Point' in mammalian cells), a cell must do three things: (1) turn on the synthesis of S- and M-phase cyclins, (2) turn off their degradation, and (3) get rid of the G1-phase CKIs. (See Fig. 1.) At the M/G1 transition ('Exit'), these three switches must be reversed. As cells proliferate, a lineage (mother-daughter-granddaughter) toggles back and forth between states of low CDK activity (G1) and high CDK activity (S-G2-M).

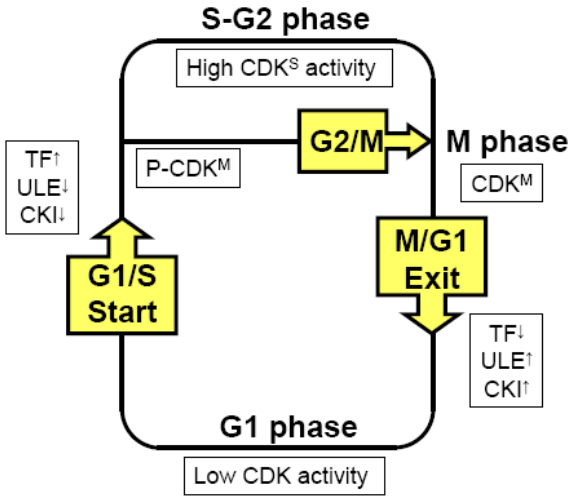

Figure 1. Three checkpoints/irreversible transitions in the eukaryotic cell cycle. G1 phase: low CDK activity. S-G2 phase: CDK-cyclin^S activity is high, CDK-cyclin^M activity is low. M phase: CDK-cyclin^M activity is high. At the G1/S transition, cyclin synthesis is turned on, cyclin degradation is turned off, and CDK inhibitors are destroyed. At the M/G1 transition, these switches are reversed. In S-G2 phase, CDK-cyclin^M activity is kept low by phosphorylation of the CDK subunit. At the G2/M transition, this inhibitory phosphorylation is removed.

Embedded inside this fundamental toggle switch is a secondary toggle switch governing the transition into M phase (see Fig. 1). During S and G2 phases, the cell is producing M-phase cyclins, but CDK/cyclin-M activity is low because of inhibitory phosphorylation of the CDK subunit. At the G2/M transition, the kinases that impose this inhibition must

be inactivated, and the phosphatases that remove this inhibition must be activated, thereby allowing the cell to transit abruptly into mitosis. At the M/G1 transition, these two switches must be reversed as well.

The scenario just described is a generic account of molecular events at the three basic transitions of the cell cycle. In any specific type of cell (e.g., budding yeast, fission yeast, mammalian cell) there may be variations on this general scheme, but most cell types studied in depth show evidence of CDK-governing toggle switches that are flipped on and off at alternating transitions of the cell cycle.

The greatest exception to this rule is the newly fertilized egg, a gigantic cell that undergoes rapid S-M cycles, lacking gap phases and checkpoints. It is arguable whether these cell cycles are governed by irreversible toggle switches or a simple periodic oscillator. Nonetheless, it is true that early embryonic cell cycles often make serious mistakes (producing polyploidy or aneuploid cells) that lead ultimately to death of the embryo. These errors seem to be a price that organisms are willing to pay in order to hurry through the most vulnerable phase of their life cycle. In this context, these errors reinforce the notion that toggle switches and irreversible transitions are crucial to maintaining genomic integrity of proliferating eukaryotic cells.

Bistability and hysteresis

From a theoretical perspective, irreversible transitions are intimately related to bistability of molecular regulatory networks. The vignette on ‘Bistability and Oscillations’ discusses the molecular mechanisms underlying bistability and hysteresis at the G2/M transition. Here we describe the molecular basis of irreversibility at Start and Exit. Figure 2 illustrates the generic interactions among CDK-cyclin, its transcription factors, its ubiquitin-ligating enzymes, and its stoichiometric inhibitors. In each case, CDK is involved in a positive feedback interaction with a ‘friend’ (TF) or a double-negative feedback interaction with an ‘enemy’ (ULE or CKI). These interactions create the possibility of bistability: two stable steady states (‘nodes’) separated by an unstable steady state (a ‘saddle point’). The stable steady states are characterized by either low or high CDK activity (Fig. 3). The CDK control system can persist in either of these stable steady states, corresponding to G1 or S-G2-M phases of the cell cycle, respectively.

For a G1 cell to commit to a new round of DNA replication and division, it must be promoted from the G1 steady state to the S-G2-M steady state. This is the job, generically speaking, of a ‘starter kinase’ (SK), typically a special cyclin-CDK pair (Cln3-Cdc28 in budding yeast, cyclin D-Cdk4/6 in mammalian cells). Transient activation of SK can induce a transition from G1 to S-G2-M. Even if SK activity disappears after the transition, the control system will remain in the stable steady state of high CDK activity. In this sense, the G1/S transition is irreversible. To exit mitosis and return to G1, the cell must transit from the high- to the low-CDK steady state. This is the job of generic ‘exit phosphatases’ (EP) that are activated during anaphase and telophase of the cell cycle. These phosphatases push ULE and CKI toward their ‘on’ states and TF toward its ‘off’ state, allowing the bistable switch to flip off. The M/G1 transition is irreversible because,

even though EP activity disappears in early G1, the control system remains in the stable G1 steady state (Tyson and Novak 2008).

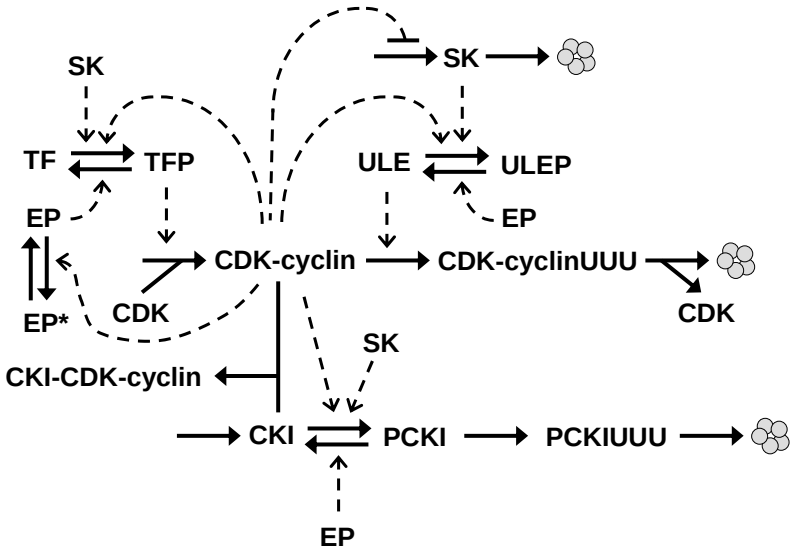

Figure 2. CDK regulatory network for a generic eukaryotic cell. Cyclin synthesis is regulated by a transcription factor (TF), and cyclin degradation is regulated by a ubiquitin-ligating enzyme (ULE). CDK-cyclin dimers can also be inhibited by binding to an inhibitor protein (CKI). CKI stability is controlled by phosphorylation: PCKI is a good substrate for a different ubiquitin-ligating enzyme, which labels CKI for degradation by proteasomes. Because TF is activated by phosphorylation by CDK-cyclin, these two components are involved in a positive feedback loop. Because ULE is inactivated by phosphorylation by CDK-cyclin, these two components are involved in a double-negative feedback loop, as are CKI and CDK-cyclin. These interactions create a bistable control system, with a stable steady state of low CDK activity (G1 phase) and an alternative stable steady state of high CDK activity (S-G2-M phase); see Figs. 1 and 3. Starter kinase activity (SK) tips the control system toward the high CDK state, and exit phosphatase activity (EP) tips the system toward the low CDK state. SK and EP are related to CDK by negative feedback loops. SK upregulates CDK-cyclin by flipping the switch to the high CDK state, but high activity of CDK-cyclin downregulates the synthesis of SK. On the other hand, high activity of CDK-cyclin activates EP, which flips the switch to the low CDK state and consequently EP is inactivated.

Irreversibility and proteolysis

For a time it was fashionable among molecular biologists to attribute irreversibility of cell cycle transitions to the proteolysis of key cell-cycle regulators at each transition. Not only are cyclins dramatically degraded at the M/G1 transition (Minshull et al, 1989), but also CKIs are degraded at the G1/S transition (Schwob et al, 1994) and CDK-inactivating kinases are degraded at the G2/M transition (Michael & Newport, 1998). As simple and appealing as this idea might be, it is clearly insufficient to explain the irreversibility/directionality of cell cycle progression.

First of all, proteolysis is not irreversible in a kinetic sense; its opposing process is protein synthesis. In general, the rates of protein synthesis and degradation will balance

each other at a unique, stable steady state of protein abundance. To upset this balance, it is necessary to switch the rates of protein synthesis and degradation between low and high values, and such switching behavior is a property of feedback interactions in a regulatory network.

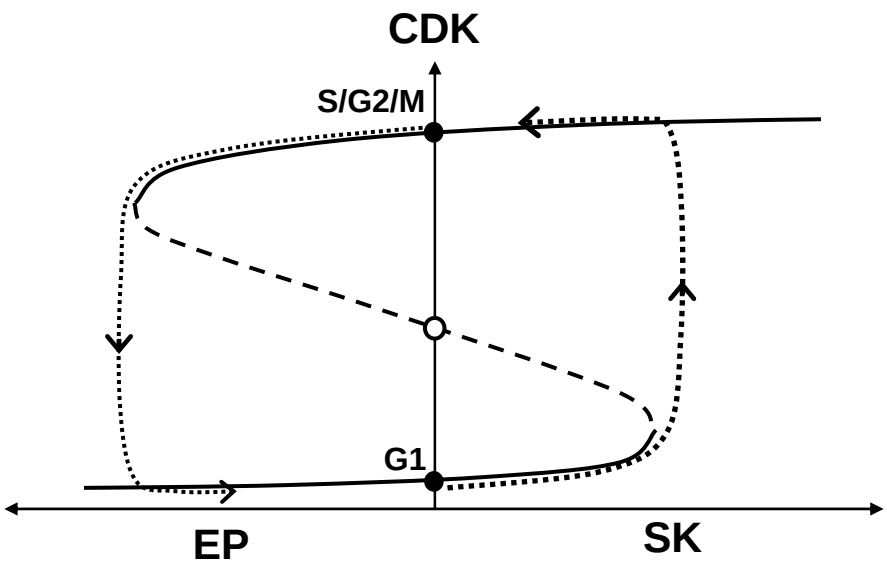

Figure 3. Bistability, irreversibility and hysteresis. The steady states of the control system in Fig. 2 are related to SK and EP activities by this bifurcation diagram. When SK = EP = 0, the control system has two stable steady states (black circles) separated by an unstable steady state (white circle). The cell can be in either G1 phase or S-G2-M phase, depending on its recent history. A newborn cell, by definition, is in G1 phase. For it to start a new round of DNA synthesis and degradation, a starter kinase (SK) must be activated. Activation of SK is controlled by checkpoint signals, such as growth factors, DNA damage sensors, size sensors, etc. Once SK flips the switch into the CDK-active state, it is no longer needed: SK activity can drop to 0, and the control system remains in the CDK-active state. For the cell to exit mitosis, undergo cytokinesis and return to G1 phase, an exit phosphatase (EP) must be activated. EP activation depends on other checkpoint signals, such as complete replication of DNA and successful alignment of all replicated chromosomes on the mitotic spindle.

In addition, it is possible, by mutations and/or drug treatments, to eliminate proteolysis at any one of the three cell cycle transitions without compromising its irreversibility. For example, *ckiΔ* mutants in budding yeast proceed irreversibly through the G1/S transition without proteolysis of any of the remaining regulatory proteins (TF and ULE). Proteasome-inhibited mammalian cells, blocked in mitosis by nocodazole, can be induced to exit mitosis irreversibly by transient inhibition of CDK activity by flavopiridol (Potapova et al, 2006). Frog egg extracts, without either cyclin synthesis or degradation, can be induced to enter or exit mitosis simply by manipulating the activities of the CDK-inhibiting kinase and/or the CDK-activating phosphatase.

In conclusion, the irreversibility of cell cycle transitions, which is crucial to maintaining the genomic integrity of proliferating cells, is a systems-level property of the molecular networks that regulate CDK activity in eukaryotic cells. Positive feedback loops in the

control system generate multiple stable steady states (G1, S-G2 and M states), and abrupt, irreversible transitions between these stable steady states are induced by pro-proliferative signals (growth factors, size increase, successful completion of prior cell cycle events) and inhibited by checkpoint signals (indicators of potentially fatal mistakes in the genome replication process). Irreversibility is not a property of any single gene, protein or reaction but rather an emergent property of systems-level interactions.

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