

Checkpoints in the Cell Cycle

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Surveillance mechanisms stop progression through the cell cycle at specific checkpoints (at the G1→S, G2→M and metaphase→anaphase transitions) if certain crucial requirements have not been met. These checkpoint controls are essential for maintaining genomic integrity and balanced growth and division.

Cell Cycle Logic

There are two requirements for successful long-term cell proliferation. One is that the steps of the chromosome replication–division cycle occur in a correct and fixed order: deoxyribonucleic acid (DNA) replication (S phase) always precedes chromosome segregation (mitosis or M phase), followed by cell division (cytokinesis). The second is that this sequence of chromosomal events repeats with a period equal to the mass doubling time (the time required to double cytoplasmic mass). The first set of constraints is necessary to maintain the integrity of the genome; the second set is necessary to maintain the cell's nucleocytoplasmic ratio within viable bounds.

The correct sequence of events is a robust characteristic of the chromosome cycle. If an early event is blocked, then usually none of the following events takes place, suggesting that cell cycle events form a dependent sequence. For example, if DNA replication is blocked by a drug or a conditional mutation, then later events – mitosis and cytokinesis – are not initiated. If mitosis is blocked, then cell division and the subsequent S phase will not take place. If cell growth is blocked by nutrient deprivation or antigrowth signals, the cell cycle will arrest in G1 or G2 (depending on cell type).

What is a Checkpoint?

Although the dependencies among cell cycle events and the coordination of growth and division have been recognized for a long time, it was not until Hartwell and Weinert (1989) introduced the concept of 'checkpoints' that the underlying regulatory principles became clear. Hartwell and Weinert hypothesized the existence of 'checkpoint pathways' by which an uncompleted cell cycle event sends an inhibitory signal to later events. They provided evidence for such pathways by studying radiation-sensitive mutants of budding yeast. In response to DNA damage caused by radiation, wild-type cells stop dividing until the damage can be repaired. In contrast, mutations in the *RAD9* gene

permit irradiated cells to divide and consequently die. Hartwell and Weinert demonstrated that these mutants are not defective in DNA repair *per se*. Rad9 (the protein product of the *RAD9* gene) is not essential for normal progression through the cell cycle but rather for the checkpoint mechanism that is responsible for blocking cell division after DNA damage.

The eukaryotic cell cycle is guarded at three checkpoints: at the G1/S boundary, the G2/M boundary, and the metaphase/anaphase boundary. Progress through the chromosome cycle can be halted at these checkpoints if the conditions for successful cell division are not met. To understand how these checkpoints work, we must first recognize the 'accelerators' and 'brakes' that control progression of the cell cycle engine, and then describe the surveillance mechanisms that sense unfavourable conditions and communicate halt signals to the engine (Figure 1).

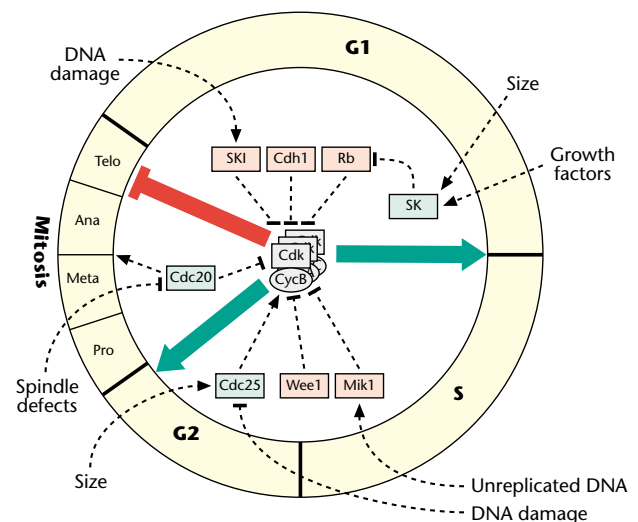


Figure 1 Accelerators (green) and brakes (red) of the cell cycle engine. Checkpoint pathways (dashed lines) modulate the activities of the accelerators and brakes. Pro, prophase; Meta, metaphase; Ana, anaphase; Telo, telophase; CKI, cyclin kinase inhibitor; DNA, deoxyribonucleic acid; Rb, retinoblastoma protein; Sk, starter kinase.

Secondary article

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Activation of a surveillance mechanism stops the cell cycle engine, allowing time for completion of a particular cell cycle event. It is traditional to use the term ‘checkpoint’ to refer to both the arrested state of the cell cycle engine and the signal transduction pathway that induces the arrested state. As expected, checkpoint pathways are essential to cell survival under adverse conditions, when cells have difficulty meeting the requirements for successful proliferation.

Although species-specific differences exist, the molecular details of cell cycle controls are amazingly conserved. Therefore, in this review, we summarize discoveries about the cell cycle engine and checkpoint controls from both yeast and metazoan cells. In general terms, we describe how the engine is accelerated in response to ‘go’ signals (appropriate cell size, proper nutrition) and how it is braked in response to ‘stop’ signals (threats to genome integrity).

The Cell Cycle Engine

The core components of the eukaryotic cell cycle engine are cyclin-dependent protein kinases (Cdks) and their regulatory subunits (cyclin). Cdks and cyclins are conserved throughout evolution (Table 1). None the less, higher eukaryotes possess more Cdk forms than lower eukaryotes. For example, there is only one essential cell cycle Cdk (generally called Cdk1) in the budding yeast *Saccharomyces cerevisiae* (Cdc28) and in the fission yeast *Schizosaccharomyces pombe* (Cdc2), whereas there are several in mammals (Cdk1–Cdk6). The same is true for cyclins: yeasts use Clb-(B type) and Cln-type cyclins, while metazoans have A, B, C, D and E types. In spite of these differences, cyclin–Cdk complexes fall into three general

classes that have different substrate specificities and therefore initiate different cell cycle events:

- G1 cyclin–Cdk complexes are important for progression through G1 phase and commitment to S phase;
- S cyclin–Cdk complexes are responsible for initiating and completing DNA replication;
- M cyclin–Cdk complexes drive eukaryotes into mitosis and restrain reentry into G1 phase.

Expression of Cdk genes plays only a minor role in regulating Cdk activity; for example, Cdk1 and Cdk2 subunits are present at constant concentrations during the cell cycle. Rather, cyclin–Cdk activity is modulated by the following mechanisms (Figure 2):

1. Cyclin availability. Association with a cyclin is absolutely required for Cdk activity. Cyclin level can be changed by transcriptional regulation and/or by specific proteolysis. Cyclins are targeted for ubiquitin-dependent degradation by the proteasome via two ubiquitin–ligase systems: SCF and APC. The SCF (Skp1–Cullin–Fbox protein complex) recognizes cyclins as substrates when they are phosphorylated, whereas the APC (anaphase-promoting complex, also called the cyclosome) ubiquitinates cyclins when specificity factors (*fizzy* and *fizzy-related*) become activated.
2. Stoichiometric inhibition. Assembled cyclin–Cdk complexes can be kept inactive by stoichiometric inhibitors (cyclin kinase inhibitors; CKIs), which form inactive trimers (CKI–cyclin–Cdk). Lower eukaryotes possess a single CycB–Cdk1 specific inhibitor (Sic1 in budding yeast and Rum1 in fission yeast), whereas higher eukaryotes utilize many inhibitors in the INK4a(p15/16/17), Cip1(p21) and Kip1(p27) families.

Table 1 Major components of the cell cycle engine

	Fission yeast	Budding yeast	Frog egg	Mammal
<i>Central kinases</i>				
Cyclin-dependent kinase	Cdc2	Cdc28	Cdc2 , Cdk2	Cdk1 , Cdk2
S and M cyclins	Cdc13, Cig2	Clb1–6	CycA, B, E	CycA, B, E
<i>Accelerators</i>				
Starter kinase	Puc1–Cdc2	Cln1,2,3–Cdc28		CycD–Cdk4
Tyrosine phosphatase	Cdc25	Mih1	Cdc25C	Cdc25C
APC auxiliary	Slp1	Cdc20	<i>fizzy</i>	<i>fizzy</i>
<i>Brakes</i>				
Cdk inhibitor	Rum1	Sic1	Xic1	p27
Cyclin degradation	Ste9	Cdh1 (Hct1)	<i>fizzy-related</i>	<i>fizzy-related</i>
Tyrosine kinase	Wee1 , Mik1	Swel	Wee1 , Myt1	Wee1
Phosphatase	Flp1	Cdc14		hCdc14
Transcription inhibitor				Rb

Proteins in the same row of the table are functionally analogous. Bolded proteins in a row are sequence homologues. APC, anaphase-promoting complex; Cdk, cyclin-dependent kinase; Rb, retinoblastoma protein.

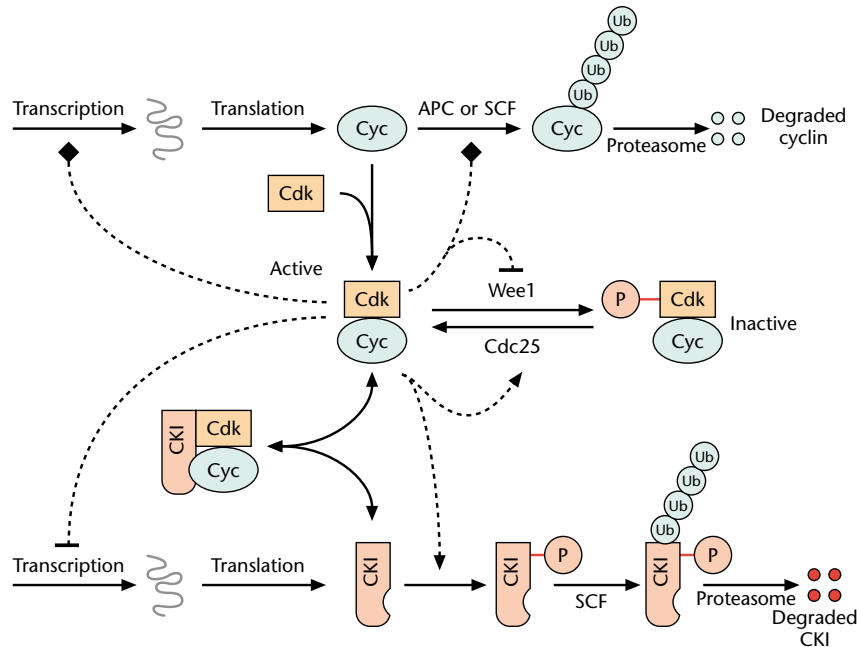


Figure 2 Three different ways to regulate cyclin-dependent kinase (Cdk) activity. Continuous arrows represent chemical transformations, while dashed arrows indicate regulatory signals (arrowhead indicates activation, blunt end indicates inhibition, diamond head indicates both activatory and inhibitory effects). APC, anaphase-promoting complex; CKI, cyclin dependent kinase inhibitor; SCF, Skp1–Cullin–Fbox protein complex; Ub, ubiquitin.

- Inhibitory phosphorylation. Cyclin–Cdk complexes can also be inactivated by phosphorylation of tyrosine and threonine residues close to the active site of the Cdk subunit. This phosphorylation is mediated by Wee1-type protein kinases, and the inhibitory phosphate groups are removed by Cdc25-type phosphatases.

These regulatory processes are modulated by the very cyclin–Cdk complexes they control, thereby creating feedback loops in the mechanism (**Figure 2**). Active Cdks phosphorylate and thereby regulate transcription factors for cyclin and CKI genes, APC components, CKI proteins and the tyrosine-modifying enzymes. A positive feedback loop is created when Cdk activates a process that has a positive effect on its activity (or inhibits a process that has a negative effect). For instance, Cdk activates Cdc25 and inhibits Wee1. By phosphorylating CKIs, Cdks promote inhibitor degradation by the SCF and thereby enhance their own activity. In a negative feedback loop, Cdk activates a process that has a negative effect on its activity. For instance, CycB–Cdk1 activates *fizzy*–APC complexes that destroy the CycB subunit.

These feedback loops create three, distinct, stable states of the cell cycle engine that correspond to the three different phases of the cell cycle (G1, S–G2 and M). (The major difference between S and G2 is the presence or the absence

of licensing factors, rather than the activities of cyclin–Cdk complexes. Licensing factors ensure that each chromosome replicates once and only once during S phase.) Progress through the cell cycle corresponds to sequential transitions from one characteristic state of the engine to the next (G1→S→G2→M→G1). The feedback loops ensure that these transitions are irreversible. Checkpoint pathways relay intracellular and extracellular signals to the feedback loops of the cell cycle engine, thereby stabilizing one phase of the cell cycle and preventing (or delaying) the transition to the next phase.

G1 Checkpoints

During G1, cells check whether their environment favours proliferation and whether their genome is ready to be replicated. This control point is called *START* in yeast and the restriction point in mammals.

START in budding yeast

START was defined by Hartwell *et al.* (1974) as the earliest genetically controlled step of the cell cycle. Yeast cells deprived of nutrient or exposed to mating pheromones before this point will arrest at *START*, whereas cells treated after *START* will finish the ongoing cell cycle and arrest in

G1 of the next cycle. Hartwell *et al.* demonstrated that the product of the *CDC28* gene was necessary for budding yeast cells to pass START. Some 14 years later, it was shown that *CDC28* encodes an essential cyclin-dependent kinase, Cdc28.

Budding yeast cells possess three unstable G1 cyclins (Cln1–3), which form complexes with Cdc28 and regulate START. These G1 cyclin–Cdk complexes act in sequence. After cells have grown to a sufficiently large size, Cln3–Cdc28 activates the synthesis of Cln1 and Cln2. Cln1–Cdc28 and Cln2–Cdc28 then promote the appearance of B-type cyclins (Clb1–6), which drives DNA synthesis and mitosis. In both nutrient-starved and pheromone-treated cells, Cln3–Cdc28 activity drops below the threshold required to activate Cln1 and Cln2 transcription. By reducing the rate of protein synthesis, starvation prevents accumulation of Cln3. Pheromone, on the other hand, activates the synthesis of a CKI (Far1) via a mitogen-activated protein kinase-like signal transduction pathway. Far1 inhibits Cln1–3–Cdc28 complexes. In summary, checkpoint signals at START in budding yeast act by blocking the activation of G1 cyclin–Cdk complexes.

Restriction point in mammals

The restriction point is the time in G1 after which mammalian cells can complete their division cycle even in the absence of growth factors or with a reduced rate of protein synthesis (Pardee, 1974). Using time-lapse video recordings, Zetterberg and Larsson (1985) discovered that, although the length of G1 varies considerably among cells, the position of the restriction point is relatively constant, occurring 3–4 h after mitosis. Cells in this time window, if they are deprived temporarily of growth factors (by serum withdrawal) or exposed temporarily to a modest concentration of cycloheximide (causing a 50% reduction in rate of protein synthesis), respond with an 8-h delay of the next cell division after the treatment is reversed. Cells beyond the restriction point do not suffer any delay of the next division. The 8-h delay ensues because growth factor-deprived or cycloheximide-treated cells stop making cyclin D (a G1 cyclin), which disappears rapidly because it is an unstable protein. When growth factor is added back (or cycloheximide is washed away), it takes about 8 h for a mammalian cell to recover the level of cyclin D necessary for sustained proliferation.

Cyclin D interacts with the mammalian cell cycle engine through the retinoblastoma protein (Rb); see **Figure 1**. Rb inhibits cell proliferation by binding to and inactivating E2F transcription factors responsible for the expression of genes encoding cyclins A and E (which are involved in DNA synthesis and cyclin B accumulation). Rb also blocks cell growth by inhibiting the transcription of ribosomal ribonucleic acid (RNA) genes. Cyclin D-dependent kinases

remove the brakes on growth and division by phosphorylating and inactivating Rb.

Irreversible transitions

Both START in budding yeast and the restriction point in mammalian cells are irreversible transitions because of positive feedback loops in the Cdk control system. In mammalian cells, G1 phase is stabilized by three effects that keep S and M cyclin–Cdks inoperative: transcription of cyclin genes is turned off (Rb active), cyclin degradation is rapid (Cdh1 active) and a stoichiometric inhibitor (p27^{Kip1}) is abundant. All three antagonists of cyclin–Cdk function can be inactivated by phosphorylation by cyclin–Cdk complexes, but, as long as their antagonists prevail, the S and M cyclin–Cdk activities remain low. The relationship between the S and M cyclin–Cdks and their antagonists creates two, alternative, self-maintaining states of the cell cycle engine: a G1 state (antagonists uppermost) and an S–G2–M state (cyclin–Cdks uppermost). It is the job of the G1 cyclin–Cdk (CycD–Cdk4,6 in mammals) to tip the scales in favour of the S/G2/M state. By phosphorylating Rb and titrating away p27^{Kip1}, CycD–Cdk4,6 complexes provoke an irreversible transition (the restriction point) to a state of commitment to DNA replication, mitosis and cell division. The transition is irreversible in the sense that, after the restriction point, cyclin D is no longer needed to keep the cell committed. Because the S and M cyclin–Cdks keep their antagonists at bay through positive feedbacks, the cell will not revert to G1 if cyclin D is lost. At exit from mitosis, all S and M cyclins are finally destroyed, the antagonists reassert themselves, and the cell is stuck in G1, unless cyclin D is present to induce another round of replication and division.

DNA Damage

Cells that meet the size and nutritional requirements commit to completing the cell cycle unless a specific threat to genome integrity is perceived. One major threat is DNA damage. Like nutrient or growth-factor sensing, DNA damage triggers a signalling network that arrests the cell cycle at specific places by inactivating Cdks (Walworth, 2000). For example, in budding yeast, DNA damage activates Rad53, which inhibits the activity of Swi4–Swi6, the transcription factor for Clns 1 and 2; hence, cells cannot pass START. In mammalian cells, ionizing radiation produces double-stranded breaks in DNA, which trigger phosphorylation and consequent activation of ATM. ATM phosphorylates a number of substrates that function in DNA repair, apoptotic death and cell cycle arrest (**Figure 3**).

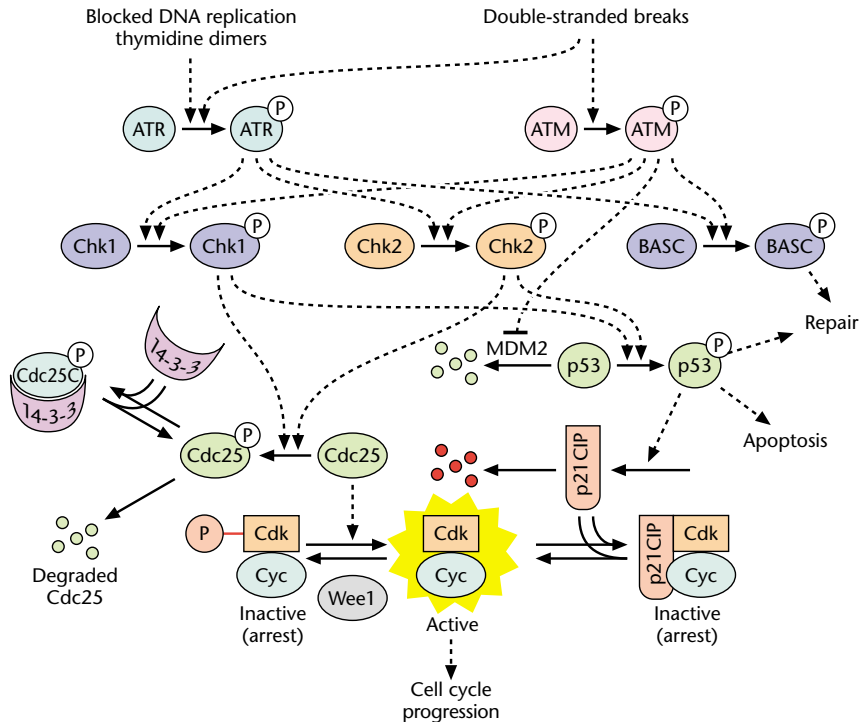


Figure 3 Mammalian checkpoint pathways that block cell cycle progression as a consequence of blocked deoxyribonucleic acid (DNA) replication and DNA damage. CIP, Cdk-interacting protein.

For instance, ATM phosphorylates and inactivates MDM2. Because MDM2 targets p53 protein for degradation by the ubiquitin–proteasome pathway, p53 accumulates in response to radiation-induced DNA damage. p53 promotes the transcription of several genes, including the gene encoding p21^{Cip1}, a stoichiometric inhibitor of all cyclin–Cdk complexes. In addition to inducing cell cycle arrest via p21^{Cip1}, p53 also induces the transcription of genes that promote DNA repair. In a process that is still not well understood, p53 can also turn on a cell suicide programme (apoptosis), presumably if the DNA damage is too great to be repaired.

In addition, ATM phosphorylates and activates protein kinases called Chk1 and Chk2, which then phosphorylate p53 and Cdc25. Phosphorylation of p53 by Chk1 or Chk2 stabilizes the protein, thereby enhancing the effects of p53 in response to DNA damage. Cdc25 protein phosphatases are activators of cyclin–Cdks (Figure 1). When phosphorylated by Chk2, Cdc25A, the G1/S phosphatase involved in activating CycA–Cdk2 and CycE–Cdk2, is rapidly degraded, further enforcing the G1-arrested state (Bartek and Lukas, 2001).

Another target of ATM is BASC (Brca1-associated surveillance complex), which associates with DNA. Activation of this complex triggers DNA repair and arrests DNA replication. By these routes, ATM plays a central

role in cell cycle arrest, genome repair and apoptosis, in response to DNA damage.

G2 Checkpoints

Progression into M phase is dependent on cyclin B-dependent kinase activity, CycB–Cdk1, also called M phase-promoting factor (MPF). Before undergoing chromosome condensation and nuclear division, cells have to be sure that their DNA is fully replicated and undamaged. If not, the cell is delayed in S–G2 by Wee1-induced phosphorylation of tyrosine and threonine residues of the catalytic subunit of MPF. (The phosphorylated dimer is called preMPF.) The G2→M transition is brought about by rapid conversion of preMPF to MPF, catalysed by Cdc25C. Hence, the obvious targets of the G2 checkpoint are the positive feedback loops controlling the activity of CycB–Cdk1 (MPF activates Cdc25C and inhibits Wee1).

DNA damage controls

DNA damage blocks the G2→M transition in fission yeast and mammals by blocking the action of Cdc25C. When Cdc25C is phosphorylated by Chk1 and/or Chk2, it binds

to the cytoskeletal protein 14-3-3 and becomes sequestered in the cytosol. Hence, it is unable to do its job on preMPF, which is nuclear. (Phosphorylation by Chk2 may also directly render Cdc25C less catalytically active.) Thus, Chk1 and Chk2 mediate cell cycle arrest in G1, S or G2 by tipping the balance toward phosphorylated, inactive, cyclin–Cdk dimers (Bartek and Lukas, 2001).

Unreplicated DNA controls

In mammals, the primary transducer of an unreplicated DNA signal (and some forms of damage such as thymidine dimers) is ATR, which phosphorylates and activates Chk1 (Figure 3). Active Chk1 promotes sequestration of Cdc25 and stabilization of p53. The results are arrest at the S–G2 checkpoint, induction of DNA repair enzymes, and possibly triggering cell suicide.

ATR and Chk1 are encoded by essential genes. Mice homozygous for deletions of either ATR or Chk1 die early in embryogenesis with high levels of DNA damage and apoptosis. Hence, ATR and Chk1 play critical roles in coordinating cell cycle events even in the absence of external threats to genome integrity.

A similar pathway is engaged in fission yeast whereby Rad3 activates Chk1 and Chk2, which phosphorylate and sequester Cdc25. Additionally, Chk2 also phosphorylates and stabilizes Mik1, a kinase that acts in parallel with Wee1. Therefore, fission yeast cells with unreplicated DNA possess reinforcing mechanisms to stabilize preMPF and block entry into mitosis.

Size control in fission yeast

Some cells check their cytoplasmic mass in G2 phase rather than in G1. In fission yeast, Wee1 plays a rate-limiting role in size control, as shown by Nurse (1975), who isolated *wee1*[−] mutants that divide at around half the size of wild-type cells. Conversely, by increasing the dosage of the *wee1*⁺ gene, Nurse was able to increase cell size at mitosis. Cdc25 is the other component that has a dramatic effect on cell size at mitosis (e.g. overexpression of *cdc25*⁺ produces the *wee* phenotype). The complex feedback signals involving Wee1, Cdc25 and Cdc13–Cdc2 (Figure 2) determine the threshold size for the G2→M transition. This threshold is modulated by upstream protein kinases (e.g. Nim1) involved in monitoring nutritional status.

Metaphase (Spindle) Checkpoint

Separation of sister chromatids during anaphase should take place only when all chromosomes are attached to the bipolar mitotic spindle via their kinetochores. The spindle checkpoint guarantees this dependence. Treating cells with microtubule-depolymerizing drugs activates the check-

point. In the presence of free kinetochores, cells do not undergo anaphase or exit mitosis, suggesting that the spindle checkpoint pathway blocks both the metaphase→anaphase and anaphase→telophase transitions. Components of the spindle checkpoint were originally defined in budding yeast, but homologues were later identified in higher eukaryotes, demonstrating that the pathway is conserved. Two independent, but similar, screens (Hoyt *et al.*, 1991; Li and Murray, 1991) for yeast mutants that do not arrest in mitosis following drug treatment identified six proteins: Bub1–3 (budding uninhibited by benzimidazole) and Mad1–3 (mitosis arrest deficient). Some of these proteins are localized at the kinetochore and some at spindle poles, the two locations generating signals for the spindle checkpoint.

The metaphase to anaphase transition

A negative feedback loop drives cells through the stages of mitosis. MPF (CycB–Cdk1) promotes the early events of mitosis (nuclear envelope breakdown, spindle assembly, chromosome alignment) and inhibits later events (Figure 4). MPF also promotes the activation of Cdc20–APC. Once activated, Cdc20–APC initiates anaphase (sister chromatid separation) by destroying a protein called securin (Pds1 – precocious dissociation of sister chromatids 1 – in budding yeast). Securin keeps a protease called separase (Esp1 in budding yeast) inactive during most of the cell cycle. By destroying the cohesin proteins that hold sister chromatids together, separase induces the first stage of anaphase. To finish the mitotic programme, Cdc20–APC also targets for degradation the B-type cyclin component of MPF. Because MPF is an activator of Cdc20–APC, when cyclin B is lost at the end of mitosis, so is Cdc20–APC activity. Hence, the activities of CycB–Cdk1 and Cdc20–APC rise and fall sequentially, as an undisturbed cell proceeds through mitosis and into G1 phase.

This negative feedback loop is the target of the spindle assembly checkpoint. Unattached kinetochores activate Mad2 protein, which then binds to Cdc20 and blocks its functions (degradation of securin and mitotic cyclins). Consequently, the cell cycle engine halts in metaphase.

The mitotic exit network

Exit from mitosis (telophase) is regulated by positive feedback loops as well; these are also targets of the spindle checkpoint. In budding yeast, the positive feedback loops arise from antagonistic relationships between the mitotic cyclins, Clb1 and Clb2, and their negative regulators, Sic1 (a stoichiometric inhibitor of Clb–Cdc28) and Cdh1 (a Clb-specificity factor for APC); see Table 1. Mitotic cyclins are not fully destroyed by Cdc20–APC, and their residual activity keeps the cell in mitosis.

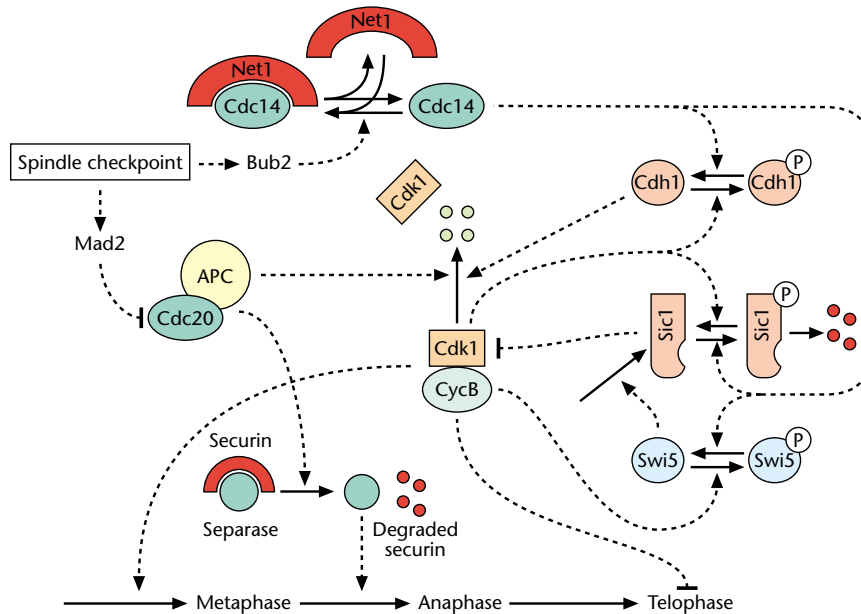


Figure 4 Checkpoint pathways that block mitotic transitions as a consequence of spindle damage. APC, anaphase-promoting complex.

Elimination of Clb–Cdc28 activity and exit from mitosis requires the upregulation of Sic1 and activation of Cdh1. However, both of these molecules are downregulated by phosphorylation by Clb-dependent kinase, and their upregulation requires a phosphatase, Cdc14. Cdc14 is located in the nucleolus in an inactive complex during most of the cycle. A signal transduction pathway, called MEN (mitotic exit network) in budding yeast, consisting of many protein kinases and a guanosine triphosphatase (Tem1), is responsible for the release of Cdc14 from the nucleolus. Released Cdc14 plays three roles: it activates Cdh1, it activates Swi5 (the transcription factor for Sic1), and it stabilizes Sic1. Release of Cdc14 from the nucleolus is controlled by the spindle checkpoint. In the absence of a spindle, Bub2 blocks the activation of the MEN and the release of Cdc14. Bub2 works by keeping Tem1 in its inactive (guanosine diphosphate-associated) form.

DNA damage-control in budding yeast

In budding yeast cells, S and M phases overlap; they have no distinct G2 phase or ‘G2 checkpoint’, and their tyrosine-modifying enzymes (Mih1 and Swe1) are not necessary for halting the cell cycle engine in response to unreplicated or damaged DNA. Rather, these surveillance signals are relayed to the metaphase checkpoint and prevent anaphase until the problems can be resolved. In budding yeast, DNA damage activates Mec1 (an ATM/ATR homologue), which then phosphorylates and activates Chk1 and Rad53, a Chk2 homologue. Activation of Rad53 results in inhibition of Cdc5, an activator of the

APC that targets B-type cyclins (Clb1–6) for degradation. Inhibition of Cdc5 results in maintenance of high Clb–Cdc28 activity, blocking anaphase and exit from mitosis. Additionally, Chk1 phosphorylates and stabilizes Pds1. Pds1 promotes attachment of sister chromatids, and its continued activity arrests cells at the metaphase to anaphase transition.

Cell Cycle Checkpoints and Cancer

Checkpoint signalling pathways arrest the cell cycle when genomic integrity is threatened, preventing the transmission of genetic mutations into subsequent cell generations. The restriction point also regulates cell cycle progression based on environmental signals (growth factors, extracellular matrix attachment, cell–cell contacts, etc.). Because of these critical functions, mutations in key checkpoint genes contribute to a variety of human diseases. Notably, most malignant cancers possess mutations in one or more checkpoint genes and are genetically unstable (Lengauer *et al.*, 1998).

Genes that normally function to prevent malignancy, such as *p53* and *Rb*, are called tumour suppressor genes. Mutations in *p53* are found in at least 50% of all malignant tumours. Loss of *p53* function could contribute to the development of cancer in several ways. (1) Cell cycle arrest in response to DNA damage would be compromised, presumably allowing a cell to accumulate additional mutations required for malignant transformation. (2) Apoptosis triggered by DNA damage and other events

(such as loss of cell–cell contact) may be compromised. (3) Telomere lengthening, which regulates cell lifespan, may be regulated by p53. Likewise, loss of Rb function would allow cells to escape the growth factor requirement at the restriction point. Loss of function mutations in ATM, Chk2 and other checkpoint proteins are also associated with tumorigenesis. Gain-of-function mutations in genes encoding proteins that advance the cell cycle (e.g. CycD, CycE, Cdc25A) are common in cancerous cells, presumably because they permit proliferating cells to escape checkpoint controls.

Even though cancers are associated with increased genetic instability, many chemotherapeutic agents function by further disrupting checkpoint signalling, to the point where malignant cells accumulate a lethal number of mutations. Disruption of Chk1 is one target of cancer chemotherapy that is having success in recent clinical trials. Continued advancements in understanding cell cycle checkpoint signals and their interaction with the cell cycle engine will foster the development of better regimens for treating cancer and other diseases of cell proliferation.

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