CELL CYCLE

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Introduction:

Cell cycle is the sequence of events that occur between two successive cell divisions. DNA replication and the division of a cell are the major events in cell cycle. Eukaryotic cell cycle is divided into four non overlapping phases – G_1 (gap1), S (synthesis), G_2 & M phase. The DNA component of the cell gets duplicated in the S phase whereas other cellular organelles are continuously synthesized in sufficient quantity to be distributed equally between the daughter cells when the parental cell is big enough to divide in the M phase. The events of chromosome separation occur during the M phase. When a cell withdraws from the process of cell division, it enters a quiescent state termed G_o phase. In eukaryotes, DNA replication and mitosis never occur simultaneously. Conversely, in bacteria the analogous process, replication and partition, are coordinated so that partially replicated chromosomes can segregate during rapid growth. All discrete events during the progression of cell are controlled at checkpoints by the regulatory proteins to insure that DNA content in the daughter cells remains constant.

Cell cycle checkpoints

The complexity of cell division and faithful distribution of genome and other cell organelles between two daughter cells demands that all the events of the cell cycle occur in a coordinated ordered sequence. The cell meets these criteria by organizing the cell cycle as a dependent series of events. Intrinsic and extrinsic cues provide signals to regulatory proteins of cell cycle which then coordinates the cell growth with the cell division. These regulatory mechanisms are termed as **cell cycle checkpoints.** The regulatory systems and feedback mechanisms senses the progression of the cell cycle to ensure that the cell does not proceed to the next step before the preceding step is duly completed, because such a premature entry into next step will be disastrous for the cell. The regulatory proteins can inhibit/delay subsequent stages in the event of failure of the previous functions or when something goes wrong. If repair is unsuccessful owing to either excessive DNA damage or genetic defects in the checkpoint machinery or the DNA repair mechanism, cells may enter senescence or undergo apoptosis. Alternatively, accumulation of DNA alterations may result in **genomic instability (GIN)** leading to cell transformation and oncogenesis.

Cell cycle checkpoints include:

Despite the recent explosion of information regarding the molecular components of cell-cycle checkpoints in eukaryotic cells, we still have only a skeletal understanding of both the identities of the DNA damage sensors and the mechanisms through which they initiate and terminate the activation of checkpoints.

A check on completion of S phase.

- **DNA damage** checkpoints. This regulatory mechanism senses DNA damage. Proliferating Cell Nuclear Antigens (PCNA) probably play a crucial role to initiate check point responses. During DNA replication, PCNA forms a homotrimeric complex that encircles DNA, creating a "sliding clamp" that tethers DNA polymerase δ to the DNA strand. Replication factor C (RFC) controls the loading of PNCA (Rad1, Rad9, and Hus1 in *S. pombe*) complex that encircles DNA at or near sites of damage to form a checkpoint sliding clamp (CSC), which probably serves as a nucleus for the recruitment of the checkpoint signaling machinery to broken or abnormally structured DNA. *(Probably monitors the Okazaki fragments & inhibits cell cycle progression until Okazaki fragments have disappeared.)*
    - before the cell enters S phase (a G_1 checkpoint);
    - during S phase, and
    - after DNA replication (a G_2 checkpoint).

During the very earliest stages of checkpoint activation, DNA damage sensors relay information, via two members of a recently defined family of phosphoinositide 3-kinase related kinases (PIKKs). In mammalian cells, two PIKK family members, are:

- PIKKs Sensor kinases
    - ataxia–telangiectasia mutated (ATM) and
    - ataxia–telangiectasia and rad3-related (ATR), and
- Checkpoint kinases
    - the checkpoint kinases CHK1 and CHK2.

Activation of these pathways in response to DNA damage results in increased levels of :

- CDK inhibitors like p21 or
- Inhibition of CDK activators such as the Cdc25 phosphatases.

These mechanisms prevent G1/S or G2/M transitions in the presence of DNA damage. Mutations in DNA damage checkpoint proteins show specific syndromes, such as ataxia–telangiectasia (*ATM* mutations), Seckel (*ATR* mutations) and li–Fraumeni (*CHK2* mutations) syndromes, all of which display increased susceptibility to cancer.

Once the genetic material is duplicated, proper chromosome segregation is controlled by the spindle assembly checkpoint (SAC) a signaling pathway that modulate CDK1 activity and prevent defects in chromosome segregation. Active CDK1– cyclin complexes phosphorylate more than 70 substrates during G2 and early mitosis to trigger centrosome separation, Golgi dynamics, nuclear envelope breakdown and chromosome condensation, among other processes. A defective SAC may provoke unequal inheritance of the genetic information that, if unrepaired, may facilitate tumor progression by accumulating numerical chromosomal aberrations (CIN).

- **Spindle checkpoints**. Some of these that have been discovered
    - detect any failure of spindle fibers to attach to **kinetochores** and arrest the cell in **metaphase**
    - detect improper alignment of the spindle itself and block cytokinesis;
    - trigger apoptosis if the damage is irreparable.

Mutations in the genes encoding checkpoint proteins often allow the abnormal cells to proceed through the cell cycle despite damage to their integrity. Such events can lead to the development of cancer.

Discovery of Checkpoint Regulatory Proteins

Experiments with mammalian cells and amphibian oocytes led to the identification of cell cycle checkpoint factors called **M-Promoting Factors / maturation promoting factor (MPF)**. Later more than 80 genes have been identified in *Sacchoromyces cerevisiae* and *Schizosaccharomyces pombe* that function during the progression of cell cycle although all of them may not involved in regulation of the cell division cycle.

Fusion	Result	**Conclusion**
S x G_1	**Both nuclei replicate**	S phase nucleus contains an S phase promoting factor
S x G_2	**S-phase cell completes replication, G2-phase nucleus waits for S-phase nucleus to complete replication and then both cells enter the M phase**	G2 nucleus cannot respond to S-phase activator (a re-replication block,) S-phase activator is also an inhibitor of mitosis
M x G_1 S or G_2	**Interphase nucleus enters precocious mitosis (regardless of state of chromosome replication)**	M nucleus contains an M-phase promoting factor
G1 x G2	Neither nucleus undergoes replication	Both S-phase and M-phase activators

	or mitosis	are present transiently

Cell cycle checkpoints can be clustered in two major groups –

- those occurring a G1 and regulating entry into the S phase
- those occurring at G2 and regulating entry into the M phase.

Different organisms attach varying degrees of significance to the G1 and G2 checkpoints. For example:

1. In yeast, *Saccharomyces cerevisiae* (where it is called START), the regulatory mechanism of the G1 assess the nutrient availability and the presence of mating pheromones before the entry into the S phase
2. In animal cells, (where it is called restriction point) presence of growth factors is necessary before the cell enters into the S phase.
3. Conversely, *Schizosaccharomyces pombe* (the fission yeast) monitors the environment in the G2 phase of the cell cycle.

Cells of both these organism senses the environment at G_1 or G2 and will arrest cell division if the environment is unsuitable. However, once checkpoint is crossed the cells get committed to complete the DNA replication, come whatever may, even if the environment becomes unsuitable to proceed with the cell division.

Many checkpoint pathways have been identified primarily through the analysis of **cdc/CDC** (cell division cycle) yeast mutants. These checkpoints are actually inhibitory pathways and possess the following features:

i) Sense mating partners ii) Coordinate cell size and cell cycle progression iii) Make nuclear division dependent upon budding	iv) Inhibit mitosis while in G1 v) Make DNA synthesis dependent upon G1 cyclins vi) Cause delay in entry from G2 and M phase and from G1 into S phase in the event of DNA damage	vii) Cause delay in separation of chromatids at anaphase until all the chromosomes are duly aligned on the spindle viii) Restrict DNA replication to once per cycle

Controlling Proteins of the Cell Cycle

The main cell cycle regulatory proteins are **Cyclin-dependent kinases** and **Cyclins**. Human cells contain multiple loci encoding CDKs and cyclins (13 and 25 loci, respectively). However, only a certain subset of CDK–cyclin complexes is directly involved in driving the cell cycle. They include three **interphase CDKs** (CDK2, CDK4 and CDK6), **a mitotic** CDK [CDK1, also known as cell division control protein 2 (CDC2)] and ten cyclins that belong to four different classes (the A-, B-, D- and E-type cyclins).

Cyclin-dependent kinases (Cdks)

- a **G_1 Cdk** (Cdk4, CDK6)
- an **S-phase Cdk** ((Cdk2)
- an **M-phase Cdk** (Cdk1)

Their levels of Cdk in the cell remain fairly stable, but each must bind the appropriate cyclin (whose levels fluctuate) in order to be activated. They add phosphate groups to a variety of protein substrates that control processes in the cell cycle.

Cyclins

- a **G_1 cyclin** (cyclin D)
- **S-phase cyclins** (cyclins E and A)
- **mitotic cyclins** (cyclins B and A)

The levels of cyclins in the cell rise and fall with the stages of the cell cycle.

The **anaphase-promoting complex (APC)**. (The APC is also called the **cyclosome**, and the complex is often designated as the **APC/C**.) The APC/C

- triggers the events leading to destruction of the **cohesins** thus allowing the sister chromatids to separate;
- degrades the mitotic cyclin B.

Cyclin Dependent Kinases (Cdks/CDKs)

Cyclin dependent kinases (**Cdks/CDK**) are enzymes that phosphorylate some key proteins that regulate the cell cycle activities. The activities of the Cdks/CDKs are in turn governed by another group of proteins known as **cyclins**. The **CDKs** are constitutively present in the nucleus in inactive form. Binding of the cyclin induces conformational changes and confers basal kinase activity and substrate specificity to the **CDKs** which can then phosphorylate one or more target proteins. **CDKs** *themselves are also regulated by phosphorylation of specific residues like Tyrosine or Threonine.*

The involvement of protein kinases or **CDKs** was actually discovered in *S. cerevisiae***.** Cell division cycle (cdc) mutants of ***S. cerevisiae*** gets blocked at **START** because of a defective gene – ***CDC28***, the product of which is a 34kD protein kinase and is the principal regulator of the G_1-S transition. In ***S. pombe***, a similar gene called ***cdc2***, encode a homologous protein kinase which helps in the transition G_2 to M phase. Genes encoding similar kinases were subsequently isolated from vertebrates, and these could restore wild-type cell cycle function to yeast cdc mutants. Significantly, the ***Xenopus*** homology of CDC28/cdc2 is **p34/Cdc2** and is a component of **MPF**, which function specifically at the G2-M transition. The similarity between yeast cdc2 and *Xenopus* Cdc2 is that both require phosphorylation at **Thr-161** for its kinase activity and the difference is that in yeast there is no **Thr-14,** instead **Tyr-15** is phosphorylated for inactivation while **Thr-14** is phosphorylated in xenopus **Cdc2.**

NOTES:

The basic concept of cell cycle, that is, that each phase of the cycle is driven by specific CDKs, has been recently challenged by genetic studies in mice. Systematic knockout of CDK loci in the mouse germline has shown that CDK2, CDK4 and CDK6 are not essential for the cell cycle of most cell types. Instead, loss of each of these CDKs results in developmental defects in highly specialized cell types.

For instance,

1. CDK4 is essential for proliferation of pancreatic β-cells and pituitary lactotrophs during postnatal development
2. Loss of CDK6 causes minor defects in cells of erythroid lineage.
3. Mice without CDK2 do not display any detectable defects in mitotic cells however, this kinase is essential for meiotic division of both male and female germ cells.
4. Only elimination of the mitotic kinase CDK1 causes cell cycle arrest, preventing embryos from developing beyond the two-cell stage.

The absence of major cell cycle defects in cells lacking individual interphase CDKs is not simply due to compensatory activities among these CDKs. Concomitant loss of multiple CDKs enhances the scope of these developmental defects but does not result in a general disturbance of the cell cycle in most cell types.

For instance,

1. Ablation of *Cdk4* and *Cdk6* results in impaired proliferation of haematopoietic precursors, leading to late embryonic death (Fig. 1). However, no defects in G1 phase or cell cycle re-entry in cells other than those of haematopoietic lineage were observed.
2. A major compensatory effect from the third interphase CDK, CDK2, has also been ruled out. Mice lacking CDK2 and CDK6 reach adulthood and do not display any defects except those observed in the single mutant strains.

Likewise, mice lacking CDK2 and CDK4 complete embryonic development and do not show cell cycle defects except in embryonic cardiomyocytes, another highly specialized cell type (Fig. 1). These observations are not unique to embryonic cells, as adult mice lacking CDK2 and CDK4 do not display obvious defects. Indeed, these mice recover normally after severe hepatectomy, indicating that adult hepatocytes proliferate normally without CDK2 and CDK4 kinases. Interestingly, there is no promiscuity between interphase and mitotic CDKs.

3. The fact that embryos lacking CDK1 do not divide in spite of carrying the full complement of interphase CDKs illustrates that these CDKs cannot compensate for the absence of CDK1. Moreover, replacement of *Cdk1* by *Cdk2* using homologous recombination also results in early embryonic lethality, indicating that CDK1 cannot be compensated for by CDK2, even when expressed from the *Cdk1* locus.

TABLE 1.

Kinase	Genotype	Phenotype refs
CDK1	*Cdk1*mut/mut	Deficiency in CDK1 results in embryonic lethality in the first cell divisions
CDK2	*Cdk2–/–*	Sterility due to defective meiosis; no effect on mitotic cells
CDK4	*Cdk4–/–*	Diabetes and defective postnatal proliferation of endocrine cells such as pancreatic β-cells or pituitary hormone-producing cells
CDK6	*Cdk6–/–*	Slight anaemia and defective proliferation of some haematopoietic cells
CDK11	*Cdk11–/–*	Embryonic lethality in peri-implantation embryos accompanied by mitotic aberrations
CDK2; CDK4; CDK6	*Cdk2–/–*; *Cdk4–/–*; *Cdk6–/–*	Deficiency in all these interphase CDKs provokes embryonic lethality by mid-gestation due to haematopoietic defects
Cdkn1b–/–	*Cdkn1b–/–*	Develop tumours . [Cdkn1b encodes for p27]
CDK2	*Cdk2–/–*; *Cdkn1b–/–*	Develop tumours with similar incidence and latency to those in *Cdkn1b*-deficient mice, suggesting the function of p27 (encoded by *Cdkn1b*) is independent of CDK2
CDK4	*Cdk4*R24C/R24C	Mice expressing an endogenous Ink4-insensitive CDK4R24C mutant develop a variety of tumour types with complete penetrance
CDK4	*Cdk4* R24C/R24C; *Cdkn1b–/–*	Mice develop aggressive pituitary tumours with short latency (8–10 weeks)

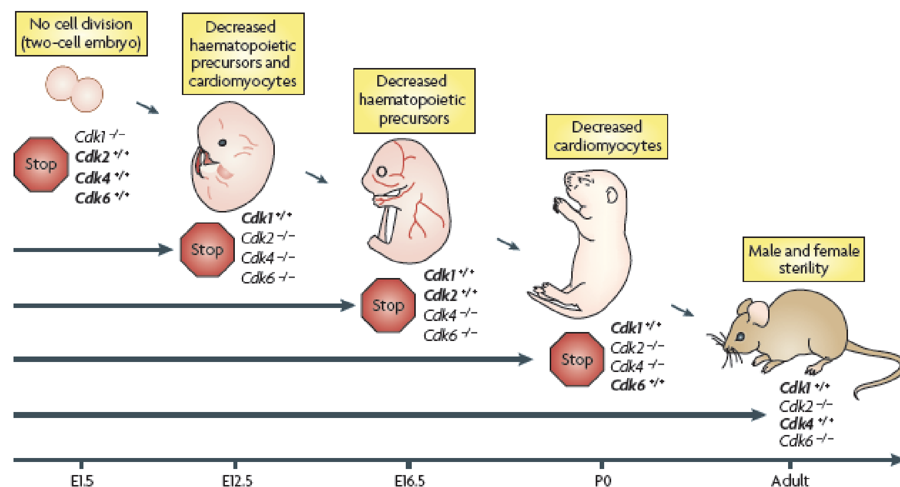


Figure 1 | Genetic interrogation of the roles of cyclin-dependent kinases (CDKs) in the mammalian cell cycle.

Cyclins and Cyclin diversity

Cyclins are a group of protein molecules which bind to different kinases (CDKs) during cell cycle progression and are responsible to induce the kinase activity. The synthesis of the cyclin molecules oscillates during the progression of the cell cycle and so also their activity. Presence of diverse cyclins confer substrate specificity to the CDKs. Change in substrate

specificity of CDKs lead to the differential expression of genes and thus facilitate the unique aspects of cell cycle control in distinct differentiated cells. Generally three types of cyclins are present in all organisms,

- **G_1 cyclins** regulate G_1-S transition (**CLN1-3** in *S. cerevisiae*, **cig1** and **cig2** in fission yeast, cyclin **C, D, E, F** in vertebrates)
- **S – phase cyclins** are required for DNA replication(**cyclins E and A** in vertebrates)
- **M – phase cyclins** control the mitosis. (**CLB1-6** cyclins in *S. cerevisiae* and **cig13** in *S. pombe*, **A & B** type cyclins in vertebrates)

All cyclins carry a conserved motif, the **cyclin box**, which is required for CDK binding. Cyclins which carry the **PEST domain** are inherently unstable undergo rapid degradation. The stable cyclins carry a **motif** called **destruction box** which is required for ubiquitination.

In vertebrates, the cyclins have been grouped into eight families designated A – H

D-type cyclins:

Ablation of the genes encoding individual D-type cyclins leads to specific developmental defects, most probably owing to their differential patterns of expression. Concomitant ablation of the three D-type cyclins results in embryonic lethality owing to haematopoietic defects similar to those observed in mice lacking CDK4 and CDK6, suggesting that the D-type cyclins are the functional activators of these G1 kinases.

E-type cyclins:

Ablation of the genes encoding cyclin E1 and cyclin E2 also results in embryonic lethality, in this case owing to specific defects in the endo-reduplication of trophoblast cells. However, these two proteins are not required for proliferation of most embryonic cells or for full development of the embryo when the placental defect is rescued. Intriguingly, CDK2 deficiency does not cause placental defects, suggesting that E-type cyclins have CDK2-independent roles, possibly in controlling DNA replication. Interestingly, expression of cyclin E1 within the cyclin D1 locus rescues most phenotypes observed in cyclin D1-null mice, thus revealing an interesting functional overlap between different classes of cyclins.

A-type cyclins:

Knockout of cyclin A2 leads to early embryonic lethality29, suggesting that the main role of this cyclin is to activate the mitotic CDK, i.e. CDK1.

B-type cyclins:

Finally, knockout of cyclin B1 also results in early embryonic death, an expected observation considering the key role of this cyclin in mitosis.

Regulation of Cdks-Cyclin activity:

The substrate specificity of CDK is conferred by cyclins. CDK activity not only requires the binding of cyclin molecules for activation but also requires phosphorylation of some target amino acid residues in the CDK molecule itself. For example, CDC28 and Cdc2 kinase in yeast require phosphorylation at Tyr-15 and Thr-161. Phosphorylation at Thr161 induces the kinase activity whereas phosphorylation at Tyr-15 inhibits the kinase activity and dominant over Thr161 phosphorylation.

In *S. pombe*, **Wee1** is the tyrosine kinase which phophorylates Tyr-15. The **phosphatase Cdc25** antagonizes the activity of Wee1 and removes the phosphate group from Tyr-15. Both Wee1 and Cdc25 are themselves regulated by intrinsic and extrinsic signals. The decision to proceed to Mitosis or arrest at G2 depends on the levels of the intrinsic and extrinsic signals and regulatory networks which feed into this checkpoint.

Apart from cyclin and phosphorylation mediated regulation, the activity of CDKs is regulated by inhibitory proteins called **CKI**s (CDK-cyclin Inhibitors). **Rum1** is a protein which is synthesized to inhibit CDK-cyclin complex and

is synthesized through out the G1 and S phase and prevents the cycle skipping DNA replication and entering mitosis prematurely. The **FAR1** is another protein in *S cerevisiae* which senses the mating pheromones and inhibits the activity of CDK cyclin complex at START thus arresting the cells at G1 for mating purpose. In animals, two families of CDK cyclin inhibitors (CKIs) are found. One blocks all CDK-cyclin activity and the other specifically inhibits D-cyclin complexes containing CDJ04 and CDJ-6.

Schizosaccharomyces pombe

In *S. pombe* control over DNA synthesis and cell division is achieved by a simple regulatory mechanism.

- Only a single type of **CDK** exists in *S. pombe*. Its regulatory activity depends on the **cyclin** it binds with.
- The CDK in *S. pombe* is **Cdc2** (Cdk1 in animals) encoded by the gene ***cdc2*** (~ to *CDC28 gene in S. cerevisiae*)
- The three cyclins are encoded by the genes ***cdc13, cig1****, and* ***cig2***. (cyclin **cdc13** is **cyclin A** in amimals)
- The **CDK** binds to different **cyclins** to form active dimmers and phosphorylates target proteins
- However, the activity of **CDK-cyclin** dimmers are also regulated by phosphorylation-dephosphorylation of certain residues on CDKs (**Tyr-15 and Thr-161**)
- Antagonistic activity of **wee-1** (**kinase**) and **cdc-25** (**tyrosine phosphatase**) regulate the phosphorylation-dephosphorylation of the **CDK-cyclin** dimmer residues
- **wee-1** & **cdc-25** respond to environmental cues or checkpoint signals and perform their antagonistic
- At the beginning of mitosis, **Cdc2** gets bound to **cdc13** cyclin (**Cdc2/cdc13**) but remains inactive because both Tyr-15 and Thr-161 remains phosphorylated
- During mitosis, **Cdc2/cdc13** becomes active because Thr-161 is only phosphorylated while Tyr-15 gets dephosphorylated
- At the end of mitosis, **Cdc2/cdc13** becomes inactive because of the degradation of Cdc13 but the phosphorylated status remains the same
- Gradually the Thr-161 is dephosphorylated and the Cdc2 returns back to its inactive form.
- After the cell enters a new G1phase, **Cdc2** binds to **cig2** to form a dimmer **Cdc2/ cig2** but the kinase remains inactive as Tyr-15 gets phosphorylated
- At some critical time, when the environment is favorable the cell decides to pass the START point and it is then that Tyr-15 on **Cdc2/ cig2** dephosphorylated and the kinase starts functioning through the G1 / S phase transition
- Delay in the initiation of S phase is seen in mutants lacking **cig2**
- In what ways the function of **Cdc2/ cig2** is down regulated is still unclear
- As new **Cdc13** is synthesized, it binds to **Cdc2** but cannot function through the subsequent G1 and S phase because of the inhibitory protein **Rum1** which is synthesized through out the G1 and S phase. **Rums1** delays the cycle and thus prevents the cycle skipping DNA replication and entering mitosis prematurely
- Mutants lacking cig2, cdc13 and cig1, exhibit complete blockage of the S phase
- However, in mutants lacking both cig1 and cig2, orderly onset of S phase and mitosis can take place suggesting that the orderly activity of **Cdc** appears to depend on the quantitative level of ***cdc13***
- Thus, cdc13 can fulfill three different functions in association with Cdc2:

- it is essential for the onset of mitosis
  - it prevents DNA replication in G2
  - it can compensates for cig2 when it lacking or mutated

- **G1/S transition** requires **Cdc2/cig2** activation but also requires **Cdc2/Cdc13** inactivation because mutation at Cdc13 has several consequences
  - fail to enter mitosis
  - there is multiple cycle of DNA replication

  These observations suggests
  - activated M phase kinases inhibits DNA synthesis
  - activated M phase kinases promotes Mitosis
  - inactivated M phase kinases allow DNA synthesis to go unchecked
  - inactivation prevents another mitosis

  This property of Cdc2/Cdc13 thus provides a checkpoint and ensures that DNA replication and cell division occur in alternate fashion.
- The target protein of Cdc2/Cdc13 phoshphorylation is probably Cdc18
- Crossing of the START point activates the transcription of Cdc18
- Cdc18 is essential for the entry into the S phase which facilitates DNA replication(over expression causes multiple DNA replication)
- Cdc18 inactivation by phosphorylation during the entry into the M phase by Cdc2/Cdc13 ensures the prevention of another S phase

Saccharomyces cerevisiae

In *S. cerevisiae* the cell division is unusual morphogenetically because of its budding type proliferative nature, where several events proceed simultaneously although the independent events are linked by a common thread. Moreover, the chromosomal segregation occurs within the intact nucleus.

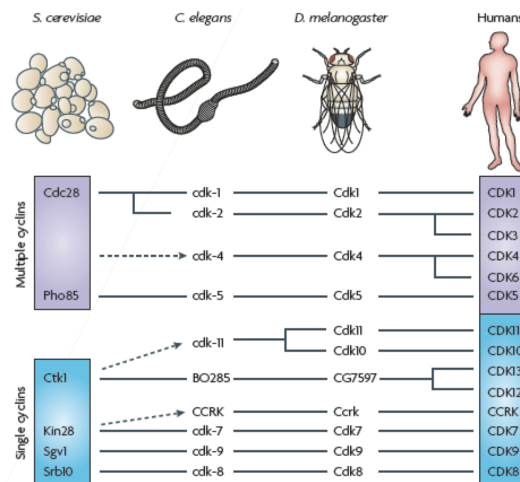
- The CDK is called **CDC28** which is equivalent to Cdc2 in *S. pombe*
- The kinase **CDC28** associates with different **G1 cyclins,** encoded by the genes **CLN**1 **CLN**2 &**CLN**3
- Mutation in any of the G1 cyclins does not stop the cell from crossing the START point but mutations at **CLN**1 **CLN**2 becomes lethal (functionally redundant genes)
- Constitutive expression of **CLN1** prevents cells from exiting mitosis
- Although the *CLN1 CLN2* **&** *CLN3* products are referred to as cyclins, they show a very weak relationship with cyclins A or B
- **G1/S transition** probably depends on the accumulation of the **CLN** proteins to certain critical value
- **CLN**3 is very unstable cyclin constitutively present in the cell. CDC28-**CLN**3 complex is also unstable. When **CDC28-CLN3** reaches a certain level, the kinase activates two transcription factors: **SBF** & **MBF (**Both SBF and MBF have a common component **SW16)**
- Activated **SBF** transcription factor initiate the transcription of two cyclins: **CLN1 CLN2**
- Activated **MBF** transcription factor initiate the transcription of **CLB5, CLB6**
- CLN1 & CLN2 form complex with CDC28 and activates six **CLB cyclins**:
  - Mitotic cyclins - **CLB1, CLB2, CLB3, CLB4**

- S phase cyclins - **CLB5, CLB6**

- All CLB cyclins share a conserved destruction box and perform the following functions
  - Firstly, they reduce the rate of proteolysis of the CLB cyclins
  - Secondly, they increase the rate of hydrolysis of a CDK-CLB cyclin inhibitor – **SIC1**
- The G_1 cyclins stabilizes the CDK-cyclin complexes at G_1 phase and aid in the transcription of S-phase cyclins, thus inducing the onset of S phase when cell growth is sufficient. However, the cell cannot pass **START** until mitosis is complete.

Animal Cells (Frogs / Mammals)

Animal cells uses similar components like the yeast cells to control the cell cycle activity but because of the inherent complexity of animal cells, they express a more diversified set of proteins required for cell cycle regulation. The number of CDKs and cyclins has increased considerably during evolution. However, only certain CDK–cyclin complexes are thought to control cell cycle progression.



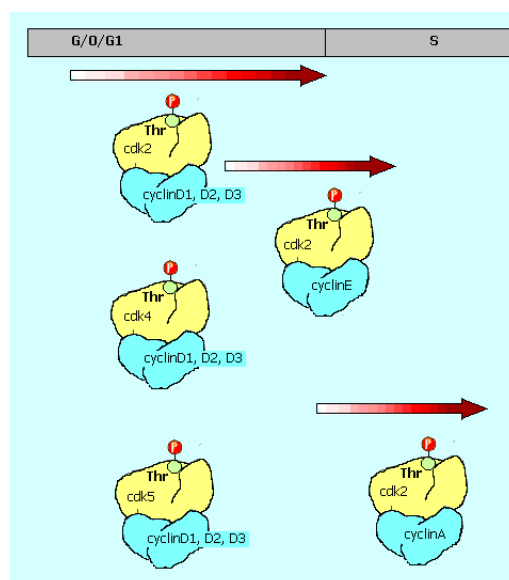
Evolution of cell cycle control: from yeast to humans

	Catalytic subunit	Regulatory subunit	Catalytic subunit	Regulatory subunit
S. pombe	Cdc2	Cig2 (Cig1)	Cdc2	Cdc13 (B-like)
S. cervisiae	CD28	CLN1-3	CD28	CLB1-4 (B-like)
Mammals / Frogs	CdK2, 4, 6	cyclins A, D1, D2, D3, E	Cdc2 / CDK1	cyclins A, B1, B2
	G1	S	G2	M

According to the 'classical' model for the mammalian cell cycle, specific CDK–cyclin complexes are responsible for driving the various events that take place during interphase in a sequential and orderly fashion. For instance:

1. G1 (preparatory phase to initiate DNA synthesis) CDKs include Cdk2, 4. These kinases associate with G1 cyclins and regulate G1/S phase transition.
2. G1 cyclins are D1, D2, D3, E, A, which are transcribed under the influence of Growth factors. Mitogenic signals trigger the expression of the D-type cyclins (D1, D2 and D3) that preferentially bind and activate CDK4 and CDK6 during G1.
3. Activation of these complexes leads to partial inactivation of the pocket proteins — rB, rBl1 (also known as p107) and rBl2 (also known as p130) — to allow expression of E-type cyclins (E1 and E2), which are required for the **G_1-S transition**

4. E1 and E2 cyclins then bind and activate **CDK2**. CDK2–cyclin E complexes activate **CDC25A phosphatase** and further inhibit rB, rBl1 and rBl2 by phosphorylate. The availability of E-cyclins during the cell cycle is tightly controlled and limited to the early stages of DNA synthesis and is believed to be essential to drive the G1/S transition.
5. **CDC25A** phosphatase in turn activate **CDK2** complexed with **cyclin A2** (cyclin A1 in germ cells) during the late stages of DNA replication to drive the transition from S phase to mitosis, a period known as the G2 phase.
6. Finally, **CDK1** is thought to be activated by A-type cyclins at the end of interphase to facilitate the onset of mitosis. Following nuclear envelope breakdown, A-type cyclins are degraded, facilitating the formation of the CDK1–cyclin B complexes responsible for driving cells through mitosis5. There are other CDKs that play minor roles in the mammalian cell cycle.
7. Both the cyclins, E and A complexes are known to phosphorylate and regulate the activity of several transcription factors including the E2F family, p53, B-Myb and the inhibitory helix-loop-helix protein Id2



D cyclins

* Growth factors stimulate the synthesis of D cyclins when a cell enters G1 from G0
* The D cyclins have a short half life and their levels decline rapidly when the growth stimulus is withdrawn
* Activity of the D cyclins is sometimes observed in later part of the G1 but not close to G1/S boundary
* The functions of D cyclins may partly be redundant but their ability to associate with different Cdks is not understood
* D cyclins probably trigger the cell in G0 to enter the G1 phase of cell cycle

Cyclins may be involved in *Licensing system*. ***CyclinB-Cdk complexes prevent Cdc6 from loading on the origin. During mitosis, Cyclin B are degraded which releases the block and allows licensing proteins to load on target sequences***

Substrates for G1 Cdk-cyclin complexes

RB gene is a tumor suppressor gene. Mutation of the RB gene causes malfunctioning of RB protein, which lead to the development of retinoblastoma tumors. The RB protein plays an important role in cell cycle activity and acts as a

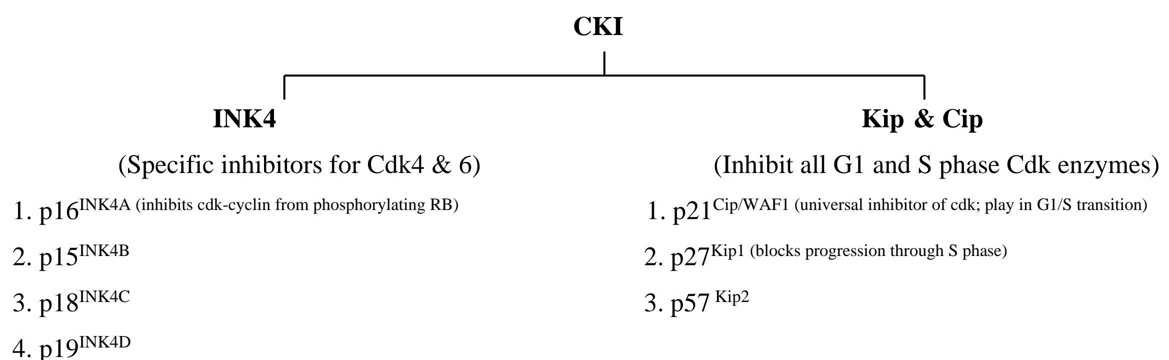
substrate of **Cdk-cyclin D complexes**. **RB** itself is a negative regulator of cell cycle. It is known to binds to ***deacetylase*** enzyme and probably removes acetyl groups from the histones at target promoters, thus inactivating the promoters.

- During the early part of **G1**, unphosphorylated **RB** binds to transcription factors of the **E2F** (transcription factor) family and inhibits transcription of genes necessary for entry into **S-phase**
- Non phosphorylated **RB** forms complexes with **Cdk4, 6, cyclin D1, 2, 3** and also with **Cdk2 cyclin-E** and activated **CDC25 - a phosphatase**.
- **G1 CDK-D cyclin** complexes phosphorylates **RB** and releases the repression of **E2F**.
- **E2F** facilitates the transcription of cyclin E which in turn activates CDC25
- CDC25 phosphatase in turn activate the S-phase specific complex Cdk2/A cyclin complex (component of DNA polymerase), which is necessary for DNA replication and S /G1 transition.

There are several transcription factors in the E2F family. RB along with two other related proteins rBl1 *(* p107) and rBl2 (p130) *bind to different members of the E2F family and suppress gene activation*

G1 Cdk-cyclin inhibitors

CDK activity is regulated by two families of inhibitors called **CKI**s (CDK-cyclin Inhibitors): INK4 proteins, (INK4A, INK4B, INK4C and INK4D) and the Cip and Kip family, composed of p21, p27 and p57.



These cell cycle inhibitors have been shown to block proliferation of adult stem cells in multiple tissue types. For instance,

- p21 and p27 may control self-renewal of neural, intestinal and haematopoietic progenitors.
- Similarly, INK4A, INK4B and INK4C are involved in modulating the self-renewal of haematopoietic, brain, lung and pancreatic stem and progenitor cells.

The expression levels of CKIs are themselves controlled by crucial pathways that modulate stem cell functionality. Loss of CKIs in stem cells is believed to contribute to tumor development. Thus, CDK4 in quiescent skin stem cells have normal CKI function but induces precocious follicular growth when this repression is relieved. Adult CDK2-deficient neural progenitors display decreased self-renewal capacity and enhanced differentiation. Information from these mouse models suggests that interphase CDKs such as CDK2 or CDK4 are modulated by CKIs to control the proliferative potential and self-renewal abilities of normal stem or progenitor cells. In human tumours, inappropriate regulation of CDKs in putative cancer stem cells may induce tumorigenesis.

Deregulation of interphase CDKs in tumours.

The mutations in CDKs (CDK4 and CDK6) and their regulators contribute to human cancer have been well defined.

- CDK4 is altered in a small set of melanoma patients by a miscoding mutation (Arg24Cys) that blocks binding of INK4 inhibitors.
- CDK6 is over expressed in some leukaemias as a consequence of nearby translocations.
- *Cdk4* and *Cdk6* are also amplified or over expressed in several malignancies (including sarcoma, glioma, breast tumours, lymphoma and melanoma).

However, the causal role of these alterations in tumour development is difficult to assess.

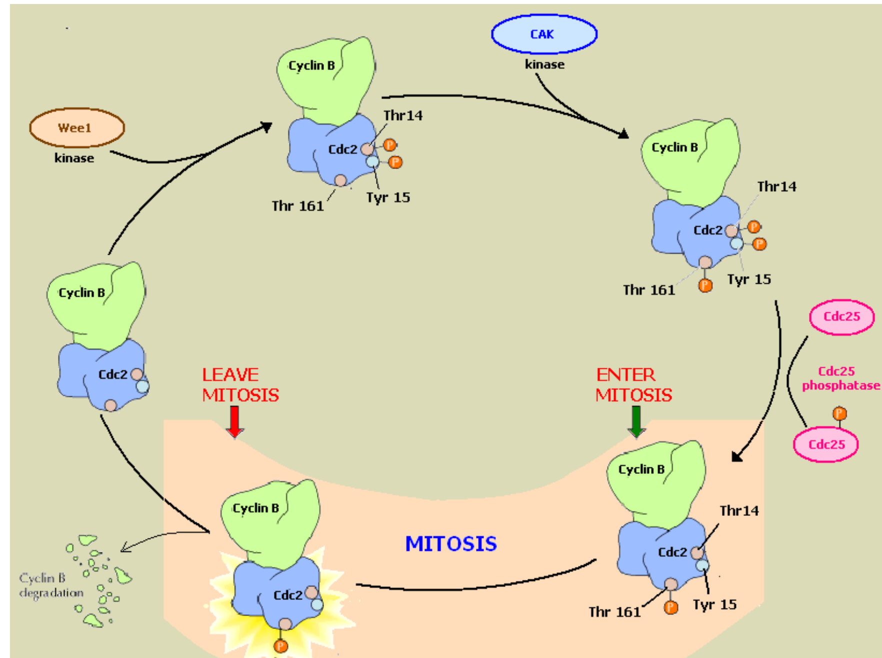
Misregulation of D-type cyclins and INK4 inhibitors is a common feature of most tumour types.

- CDK6 kinases are hyperactive in human cancer with preference for CDK6 in mesenchymal tumours (leukaemias and sarcomas),
- CDK4 in epithelial malignancies (in endocrine tissues and mucosae) and in some sarcomas.
- *Cdk2* has not been found mutated in human cancer.

However, E-type cyclins are often over expressed in human tumours, and expression of the p21 and p27 inhibitors is frequently silenced during tumour development. So far there are no models for CDK6- or CDK2-induced tumorigenesis. As p21 and p27 also inhibit CDK1, it is possible that deregulation of CDK1 activity might be responsible for tumour development in those malignancies lacking p21 or p27 expression.

G2/M Transition

- In Higher eukaryotes, the G2/M transition is better understood as a universal control mechanism seems to regulate the entry into the M-phase, which is common to all eukaryotic cells.
- The protein kinase activity of Cdc2/CDK1 ($p34^{cdc2}$) lead to the major events in the M-phase
- High levels of Cdc2/CDK1 is maintain in the cell during the M-phase that activates the key proteins required for the M phase by way of phosphorylation
- Cdc2/CDK1 complexes with Cyclin B for prior to activation. Cyclin B degradation is required for the cell to exit the M-phase.
- The activation of Cdc2/CDK1 is associated with the dephosphorylation of the phosphorylated tyrosine 15 and threonine 14 residues and phosphorylation of Thr 161 by CAK (Cdc2 activating kinase).
- Eukaryotes possess a large number of *Cdc2* genes (~ 10 genes) related to cdc2 homologue. It is not clear how many *Cdc2* genes are transcribes and are functional and also whether they participate in other cell cycle activities
- The figure below shows the functioning of Cdc2-cyclins in animals which is similar to that found in yeast cells.
- $p13^{suc1}$ interacts with $p34^{cdc2}$ and may be involved in its rephosphorylation at the end of the M-phase.



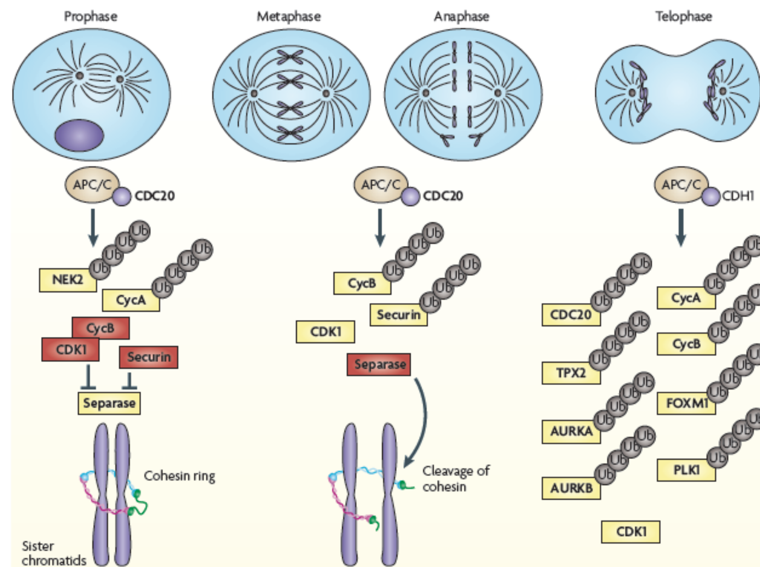
- The phosphorylation of Thr-161 is carried out by **CAK**
- The substrates of the Cdc2 protein kinases are proteins involved in the maintenance of the cell in the G2-phase. The phosphorylation of these proteins may change their functions and permit the cell to enter the M-phase
- The key substrates of Cdc2 protein kinase are:
  - **Histone H1** - the phosphorylation of this protein may be important for chromosomal condensation to occur
  - **p60*src*** - phosphorylation of the mitotic-specific sites of this protein may influence the cytoskeleton and lead to changes in the cell shape and other **DNA binding proteins** that need to be released for chromosomal condensation to occur
  - **Lamin** - this is a protein associated with the nuclear envelope
  - **Centrosomal protein** - these proteins are associated with centrioles, the organizing center of the cell for microtubules associated with the cytoskeleton
- Activation of the MPF drives the cell through the M phase committal point, which leads to the following events necessary for cell division:
  1. chromosomal condensation
  2. cytoskeletal reorganization
  3. nuclear envelope breakdown
  4. cell shape changes

Anaphase-promoting complex (APC/C)

Once chromosomes are condensed and aligned at the metaphase plate, CDK1 activity is switched off to allow sister chromatid separation through activation of separase (also known as separin), a protease that cleaves the cohesin subunit SCC1 (also known as rAD21), eliminating chromatid cohesion. Inactivation of CDK1 is also required for chromosome decondensation, re-formation of the nuclear envelope and cytokinesis74. Inactivation of CDK1 is accompanied by activation of Cdc14 proline-directed phosphatases, and possibly other phosphatases such as PP1 or PP2, to remove CDK1-dependent phosphates from mitotic substrates.

- **M-phase promoting factor** activities gradually drives the cell to enter **metaphase** of mitosis. At this point, the **anaphase-promoting complex** (**APC/ or Cyclosome**) gets activated. Two separate regulatory proteins (**CDC20, Cdh1**) complex with APC to carry out the functions during mitosis.
- **APC-CDC20** is a large complex of 8 subunits activated during the mitotic phase. It functions as an E3 ubiquitin ligase that binds to the target proteins and marks it with ubiquitin. The ubiquitinated substrate is then degraded by the proteosomes.
    - The first event of **APC-CDC20** is to targets cyclin A and NEK2 for degradation by ubiquitylation In the presence of unaligned chromosomes, **separase** is kept inactive by **securin** and **CDK1–cyclin B**. Under these conditions, sister chromatids are held together by **cohesins**. After complete bipolar attachment of chromosomes to the mitotic spindle, cyclin B and securin are also ubiquitylated by APC/C–CDC20, but in a SAC-dependent manner.
    - This triggers destruction of **cohesins** thus allowing the sister chromatids to separate;
        - **Cohesins** associates with sister chromatids during S-phase and keep them togather
        - **Separin,** another protein has the ability to releases **cohesins** and free the sister chromatids to move toward the poles
        - **Pds1p** activates **separin** but sequesters and keeps **separin** in an inactive state.
        - **Pds1p** degradation activates **separin** which degrades **cohesins** and thus facilitates metaphase-to-anaphase transition i.e. the separation of sister chromatids & their movement toward the poles (= anaphase) & complete mitosis
    - The second event to occur is the degradation of **Cyclin B** by **APC/C–CDH1** to inactivate the **M phase kinase** so that the cell can exit from mitosis.
    - Inactivation of Cyclin B occurs by ubiqutination
    - Degrades **geminin**, a protein that has kept the freshly-synthesized DNA in S phase from being re-replicated before mitosis
    - Turns on synthesis of G_1 cyclin for the next turn of the cycle;
- Two separate regulatory proteins (**CDC20, CDH1**) of **APC** follow separate route to inactivate particular substrate.
    - Binding of **CDC20** to APC allows the degradation of **Pds1p** specifically, which controls the transition from metaphase to anaphase
    - Binding of **CDH1** allows APC to degrade **Clb2 (Yeast cyclin B)**, necessary to exit mitosis.
    - CDC20 is regulated by two different genes – ***Mad*** and ***Bub***. Mutation at these two locus causes aberrant segregation of chromosomes. Mad proteins bind to **CDC20** and prevent it from activating the **APC**. Proper alignment of all kinetochores releases the inhibitory effect of Mad proteins on CDC20, which can now activate the APC
    - *APC/C–CDH1 substrates involved in mitotic progression include CDC20, TPX2, forkhead box protein M1 (FOXM1), aurora kinase A (AURKA), AURKB and PLK1. Additional APC/C–CDH1 targets involved in the control of DNA replication, such as SKP2, CDC6 or geminin, are omitted for clarity*

Some cells deliberately cut the cell cycle short allowing repeated S phases without completing mitosis and/or cytokinesis. This is called **endoreplication** and is observed embryonic development of *D. malanogaster*



An overview of key molecules involved in mitotic progression and chromosomal instability.

The spindle assembly checkpoint (SAC).

SAC is a signaling pathway that ensures proper segregation of sister chromatids by inhibiting the metaphase–anaphase transition until all chromosomes are bipolarly attached to the mitotic spindle. Briefly, chromosome segregation is mediated by the anaphase-promoting complex/cyclosome (APC/C), an E3 ubiquitin ligase responsible for targeting mitotic cyclins, among other mitotic regulators, for degradation. Target selection is mediated by the APC/C cofactors, CDC20 or CDH1 (also known as FZr1). CDC20 is the key APC/C cofactor of this pathway: it targets securing (encoded by *PTTG1*) and cyclin B for degradation once attachment is complete. Cyclin B is an obligatory target for APC/C to switch off CDK1 activity during anaphase. If CDK1 activity is recovered after chromosome segregation, chromosomes re-condense and cells are not able to exit from mitosis. CDC20 is also crucial for targeting cyclin A and NEK2 for degradation, although this activity is independent of SAC. After anaphase, APC/C function is maintained by CDH1, a cofactor that mediates the ubiquitylation of a number of mitotic (cyclin A, cyclin B, TPX2, aurora kinases A and B, PlK1 and CDC20 itself) and DNA replication (CDC6, geminin and the F-box protein SKP2) proteins, preventing CDK1 activation during exit from mitosis and G1 phase of the following cell cycle.

EXIT FROM MITOSIS

- Exit from mitosis requires the activation of the key phosphatase **Cdc14**.
- During interphase, Cdc14 remains sequestered within the nucleolous (bound to nucleolar proteins)
- **Cdc14** has two substrate – a) **APC^{Cdh1}** b) **Sic1**
- Dephosphorylation of **Cdh1** activates **APC^{Cdh1}**
- Dephosphorylation of **Sic 1** enables it to inactivate mitotic cyclins
- What activates the release of **Cdc14** from the nucleolus? Two factors – **Tem1** and exchange factor **Lte1** seems to function in the release of **Cdc14**. Tem1 when bound to GTP is active and when bound to GDP is inactive. Lte1 is responsible to replace GDP with GTP.

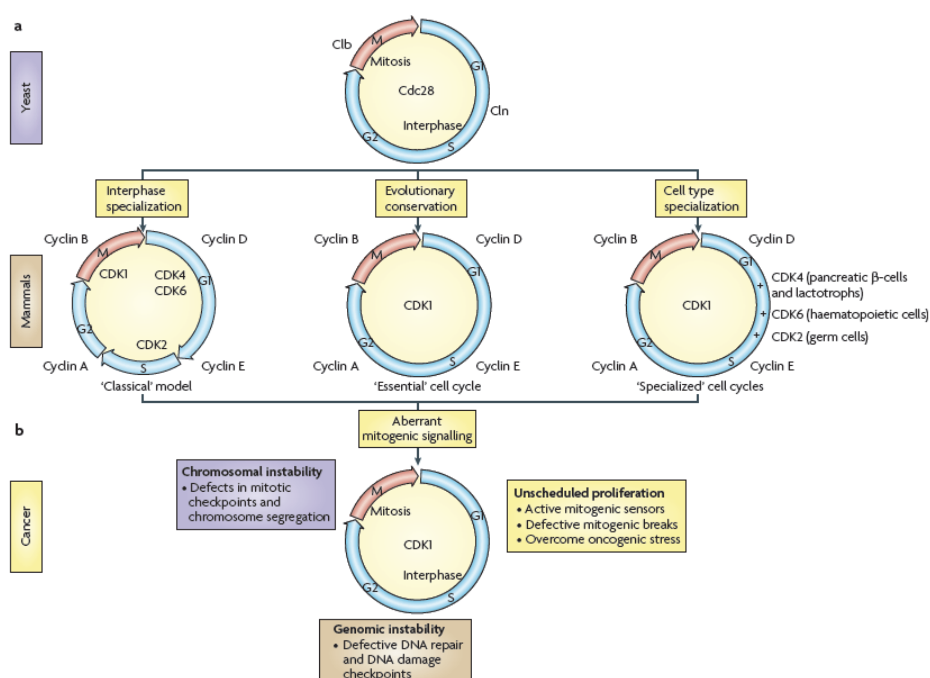
Thus, there seems to be present several parallel pathways in cell cycle control, which lead to the same out come – in this case the inactivate the mitotic kinase by dephosphorylation.

Arguably, the most striking result derived from genetic interrogation of the mammalian cell cycle came from the observation that mouse embryos develop normally until mid gestation without all interphase CDKs (FIG. 2). Although

these embryos fail to thrive owing to defective haematopoiesis, none of the other cells display obvious cell cycle defects, except for cardiomyocytes, which appear in decreased numbers. As early development is the period of most active cell division and when organogenesis takes place, these observations imply that CDK1 is sufficient to drive cell division in most cellular lineages. These observations do not imply that interphase CDKs do not contribute to normal cell cycles even in those cells in which they are dispensable. However, the fact that CDK1 alone can drive the cell cycle in most mammalian cells suggests that the mammalian cell cycle is not conceptually too different from that of yeast (FIG. 1).

CELL CYCLE AND CANCER

Tumour cells accumulate mutations that result in constitutive mitogenic signaling and defective responses to anti-mitogenic signals resulting in unscheduled proliferation. In addition, most tumours acquire **genomic instability** (GIN) that leads to additional mutations as well as **chromosomal instability** (CIN), a defect responsible for numerical changes in chromosomes. These alterations, taken together, result not only in proliferative advantages but also in increased susceptibility to the accumulation of additional genetic alterations that contribute to tumour progression and acquisition of more aggressive phenotypes. These three basic cell cycle defects — unscheduled proliferation, GIN and CIN — are mediated, directly or indirectly, by misregulation of cyclin-dependent kinases (CDKs). However, this hypothesis needs to be validated by experimental evidence. regardless of the function that interphase CDKs may have within the normal cell cycle, these observations have raised an important issue: do tumour cells retain the same CDK requirements as the normal cells from which they originate or do they acquire specific needs for CDK activity during tumour development?



Tumour associated mutations frequently deregulate certain CDK–cyclin complexes, resulting in either continued proliferation or unscheduled re-entry into the cell cycle. Cell cycle checkpoints that sense possible defects during DNA synthesis and chromosome segregation through modulation of CDK activity arrests cell cycle progression and allows cells to properly repair these defects, thus preventing their transmission to the resulting daughter cells.

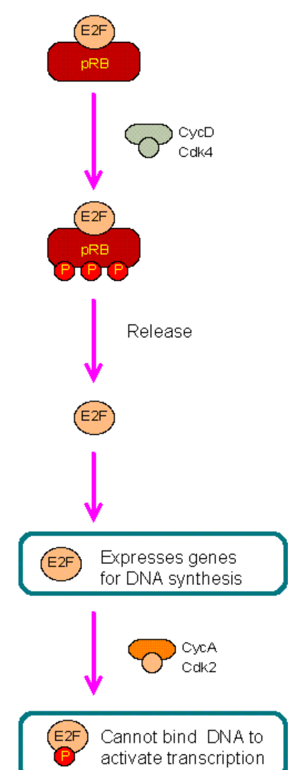
The DNA damage checkpoint protects cells from the constant attack by exogenous as well as endogenous genotoxic agents (for example, chemicals, free radicals, ionizing radiation, by-products of intracellular metabolism or medical therapy) that induce diverse alterations in the DNA molecule. These alterations are sensed by a signaling pathway that ultimately leads to cell cycle arrest. If repair is unsuccessful owing to either excessive DNA damage or genetic defects in either the checkpoint or the DNA repair machinery, cells may enter senescence or undergo apoptosis. Alternatively, accumulation of DNA alterations may result in GIN leading to cell transformation and oncogenesis. Once the genetic material is duplicated, proper chromosome segregation is controlled by the spindle assembly checkpoint (SAC), a signaling pathway that modulates CDK1 activity and prevents defects in chromosome segregation. A defective SAC may provoke unequal inheritance of the genetic information (FIG. 1) that, if unrepaired, may facilitate tumour progression by accumulating numerical chromosomal aberrations (CIN).

In addition, recent studies have proposed that the DNA damage response may act as an anticancer barrier. Proliferative forces such as activation of the Ras, MYC or E2f signalling pathways induce DNA damage-responses. It is therefore not surprising that human tumour cells often show constitutive activation of DNA damage signalling pathways, represented by activated ATM, CHK1 or CHK2 kinases, phosphorylated histone H2AX (γH2AX) and p53, all of which are markers of a DNA damage response.

Moreover, activation of the DNA damage response is at its maximum level in the early, pre-invasive stages of human tumours and correlates with the presence of senescence markers. These markers precede the deregulation of the DNA damage response pathway by specific mutations in its regulators, mainly the ATM–CHK2–p53 pathway. Under these conditions, CDKs become hyperactive owing to the reduced expression of their CDK inhibitor p21 in the absence of p53, its primary transcriptional activator. Likewise, CDKs also become hyperactive by constitutive activation of Cdc25 phosphatases, which eliminate inhibitory phosphorylation sites on CDK molecules. These alterations, taken together, result in cell cycle progression in the presence of damaged DNA, leading to increased GIN (FIG. 1). To what extent the hyperactivation f CDKs may participate in GIN by directly deregulating DNA repair is unknown at present.

Initiation and Termination Mechanism of S phase

DNA replication occurs in the S phase of the cell cycle. DNA replication in mammals is triggered by the expression proteins, activated by the transcription factor **E2F** which, in turn, is regulated by the protein **pRB.** When pRB binds to E2F, it will inactivate the transcriptional function of E2F, thereby inhibiting DNA replication. With such a critical role, pRB is known as the "**master brake of cell division**".



The Initiation and termination process of the S phase. During G_0 or early G_1 phase, E2F is inactivated by pRB. In late G_1 phase, the Cyclin D-Cdk4 complex phosphorylates a specific set of sites on pRB, resulting in the release of E2F, which then leads to the production of proteins needed for DNA replication. E2F also stimulates the production of Cyclin E, cyclin A and E2F itself. The Cyclin E-Cdk2

complex can also phosphorylate pRB, thus accelerating the cell cycle advance into the S phase. On the other hand, the Cyclin A-Cdk2 complex can phosphorylate E2F, abolishing its transcriptional function. Consequently, DNA replication is terminated. It is interesting to note that the Cyclin E-Cdk2 complex cannot phosphorylate E2F. Otherwise, the DNA replication would be terminated prematurely.

ATM

ATM (="**a**taxia **t**elangiectasia **m**utated") gets its name from a human disease of that name [Link], whose patients — among other things — are at increased risk of cancer. The ATM protein is involved in

- detecting DNA damage, especially double-strand breaks;
- interrupting (with the aid of p53) the cell cycle when damage is found;
- maintaining normal telomere length.

MAD

MAD (="**m**itotic **a**rrest **d**eficient") genes (there are two) encode proteins that bind to each kinetochore **until** a spindle fiber (one microtubule will do) attaches to it. If there is any failure to attach, MAD remains and blocks entry into anaphase. Mutations in *MAD* produce a defective protein and failure of the checkpoint. The cell finishes mitosis but produces daughter cells with too many or too few chromosomes (aneuploidy). Aneuploidy is one of the hallmarks of **cancer cells** suggesting that failure of the spindle checkpoint is a major step in the conversion of a normal cell into a cancerous one. Infection with the **human T cell leukemia virus-1** (**HTLV-1**) leads to a cancer (**ATL** = "adult T cell leukemia") in about 5% of its victims. HTLV-1 encodes a protein, called **Tax**, that binds to MAD protein causing failure of the spindle checkpoint . The leukemic cells in these patients show many chromosome abnormalities including aneuploidy.

Wee mutants allows precocious transition of cell size checkpoints

cdc mutants are blocked at various stages of the cell cycle, yet continue to grow.

Gene (budding yeast)	Gene (fission yeast)	Gene product	Loss of function in mutants
*CDC*2	*pol*3	Catalytic subunit of DNA polymerase δ	Arrest in S phase
*CDC*9	*cdc17*	DNA ligase	Arrest in G_2 with imperfectly replicated DNA
*CDC*29	*cdc*2	Serine/threonine protein kinase	Arrest at start or at mitotic entry (G_2) checkpoint
SW16	*cdc*10	Gene regulatory protein required for transcription of G_1 cyclins	Failure to enter S phase
CLN 1,2,3,	*?*	G_1 cyclins	Arrest at 'start' (if all three genes are inactive)
CLB 1,2,3,4	*cdc*13	Mitotic cyclins	Arrest at mitotic entry(G_2)
*WEE*1	*wee1*	Tyrosine protein kinase	Premature passage past mitotic entry (G_2) checkpoint, hence small size
*MIH*1	*cdc25*	Tyrosine protein phosphatase	Arrest at mitotic entry (G_2) checkpoint
*RAD*9	*?*	Protein of unknown function	Failure to delay mitosis when DNA is damaged (loss of feedback control)
*DIS*2, *S*1	*dis2*	Protein phosphatase 1	Arrest mitosis

.

The following table presents the mutants, the product of these genes and their role in M-phase entry.

Gene	Protein	Function
cdc2	p34^{cdc2}	a serine-threonine protein kinase of 34,000 daltons that complexes with cyclin to form the MPF; the inactive form of the protein is phosphorylated at threonine (T) and tyrosine (Y) residues; the phosphorylation appears to performed by p60^{src} in humans; the active form of the protein is dephosphorylated and it functions by phosphorylating a number of proteins; this phosophorylation activity is coupled to the entry into the M-phase; the protein must be associated with a normal cyclin protein for the M-phase to be completed normally; association with deletion mutants of cyclin halts the M-phase before it is completed
cdc25	p80^{cdc25}	a protein of 80,000 daltons that assists with the dephosphorylation of p34^{cdc2} by either inhibiting its phosphorylation or promoting its dephosphorylation; its concentration increases as the cell approaches the M-phase suggesting the accumulation of this protein to a specific concentration is required to activate p34^{cdc2} ; its increase in concentration appears to be coupled with the completion of the S-phase
suc1	p13^{suc1}	a protein of 13,000 daltons which may be involved in the inactivation of p34^{cdc2} late in mitosis by inhibiting its kinase activity or promoting its phosphorylation