Cell Cycle Clock

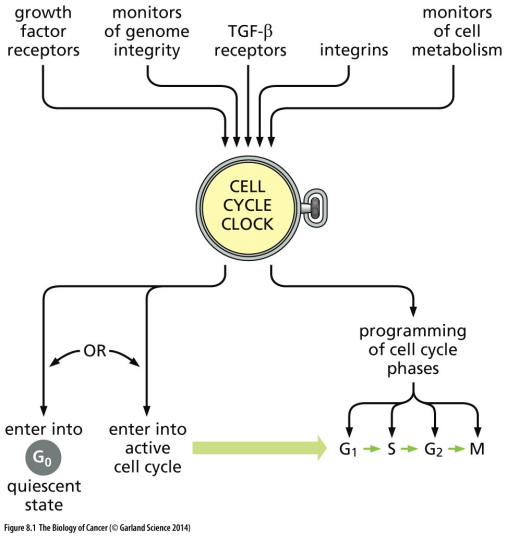


Figure 8.1 The Biology of Cancer (© Garland Science 2014)

- The fate of individual cells thoughout the body is dictated by the signals that each receives from its surroundings
- Mitogen growth factors are required for proliferation
- Other signaling proteins, transforming growth factors β (**TGF- β**) force proliferation
- Extracellular signals may persuade a cell to enter into a **post-mitotic**, differentiated state from which it will never re-emerge to resume proliferation

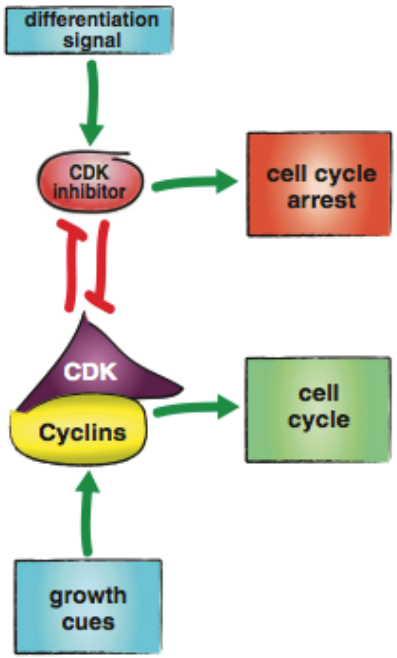
- **The master governor** that receives a wide variety of incoming signals and makes major decisions concerning the fate of the cell: **the Cell Cycle Clock**

The cell cycle clock is a network of interacting proteins that receives signals from various sources both outside and inside the cells, integrates them and decides the cell fate.

It decides in favour of proliferation (cell cycle and division) or in favour of quiescence, a non proliferative state of the cell

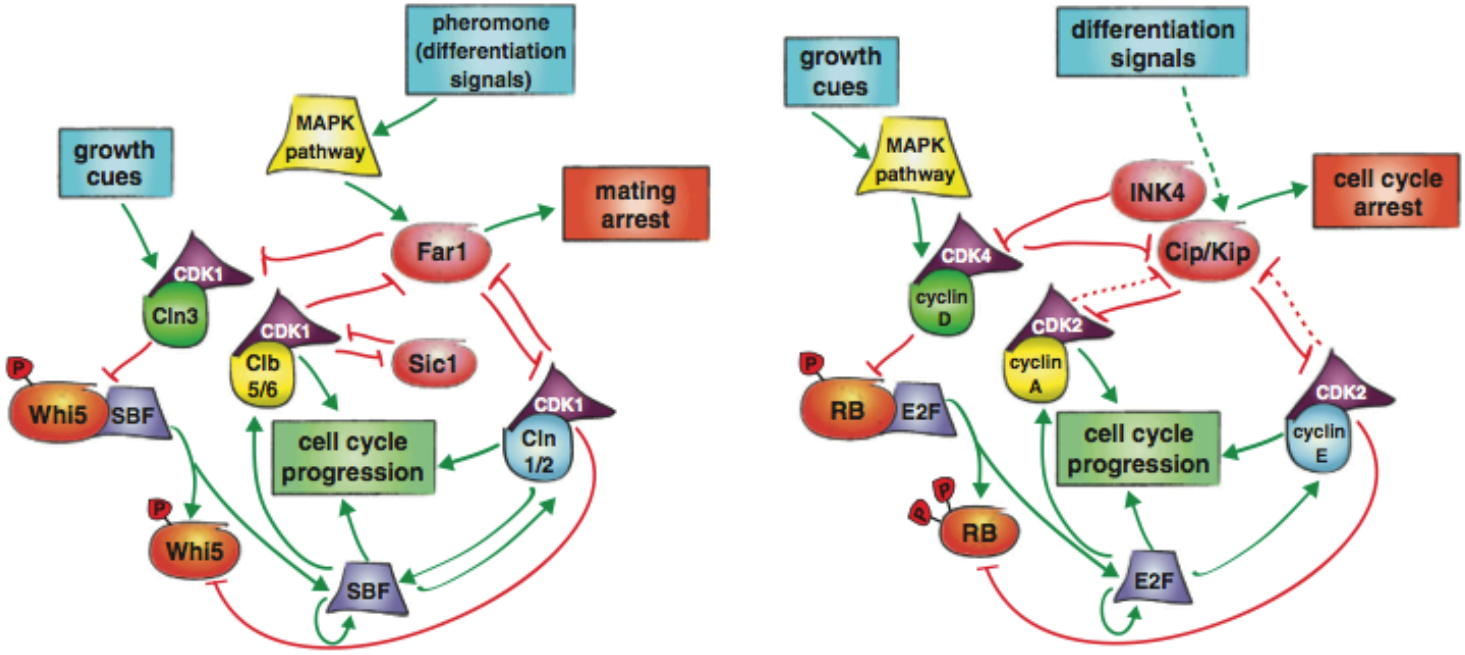
Most cells withdraw from the cell cycle during G1, entering the G0 state, to differentiate. Some differentiated cells (*i.e.* fibroblasts and lymphocytes) can be stimulated to reenter the cycle and replicate

Commitment to cell division



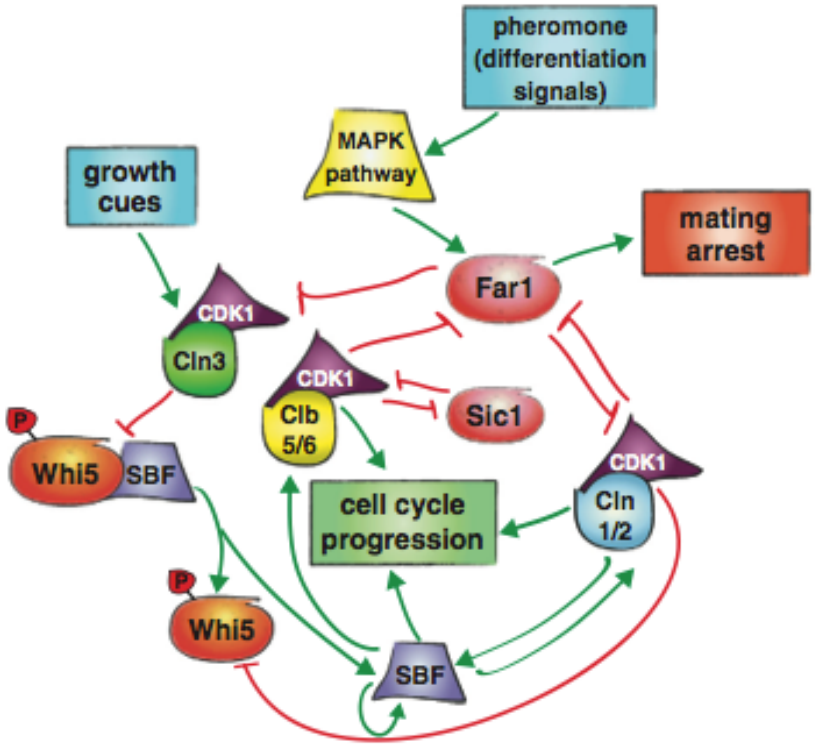
- In both mammals and yeast, input signals of diverse origin increase CDK activity to reach a threshold level driving commitment
- The transcriptional inhibitor Whi5 and Rb as well the CKI proteins are involved in the down-regulation of CDK activity
- The low-CDK state is destabilized by an increasing input signal leading to rapid cell cycle entry
- Input signals raise cyclin-CDK levels until they traverse a threshold beyond which positive feedback becomes self-sustaining

Conservation of G1 control networks



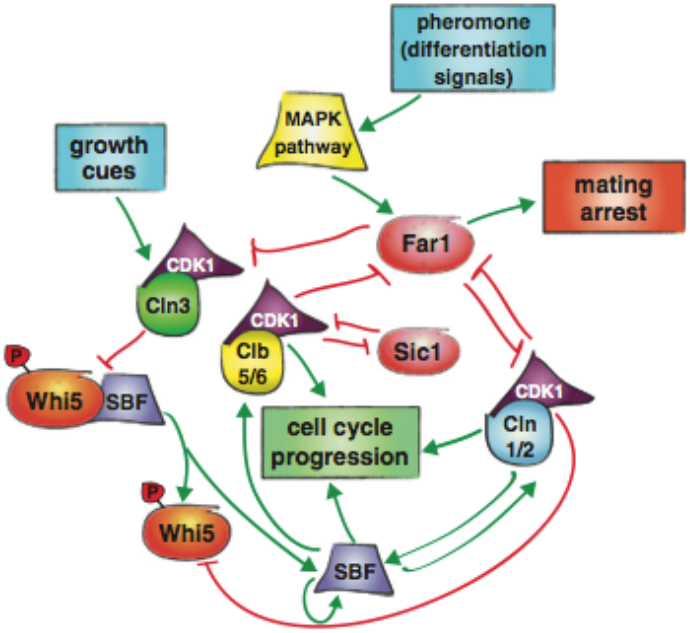
Signaling leads to an increase in cyclin-dependent kinase (CDK) activity via cyclin synthesis, which is largely responsible for promoting progression into S phase

Budding yeast



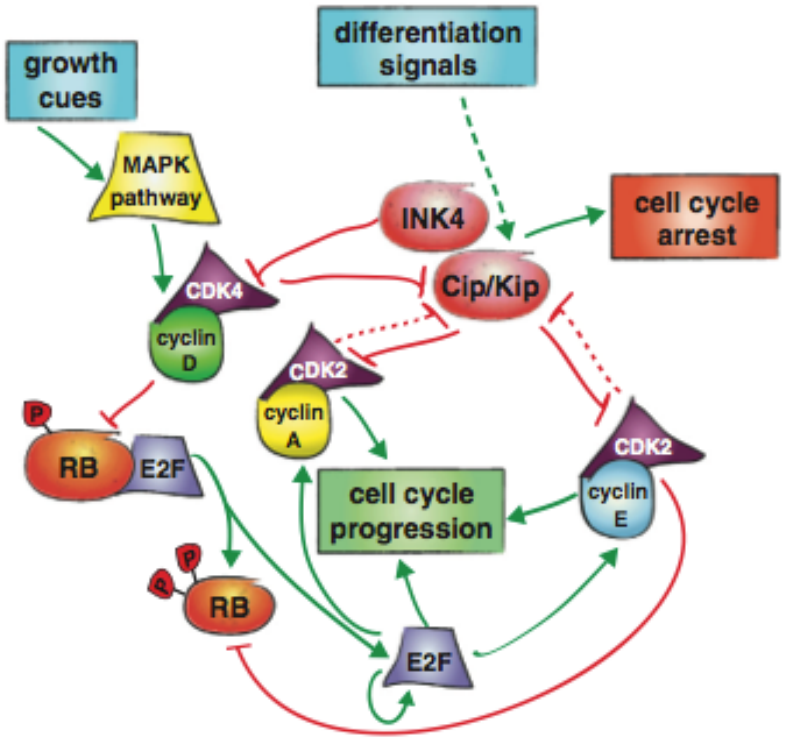
- Before CDK activation, budding yeast spends variable amounts of time in G1, with smaller cells generally taking longer to reach Start.
- This size-dependent progression functions primarily in daughter cells and requires that growth be coupled to the cell cycle. One likely coupling mechanism would rely on increasing levels of a cell cycle-regulating 'sizer' protein whose rate of synthesis is proportional to the overall protein production rate.
- A good sizer candidate in budding yeast is the G1-S activator Cln3, which drives progression through Start in a dosage-dependent manner.

Budding yeast



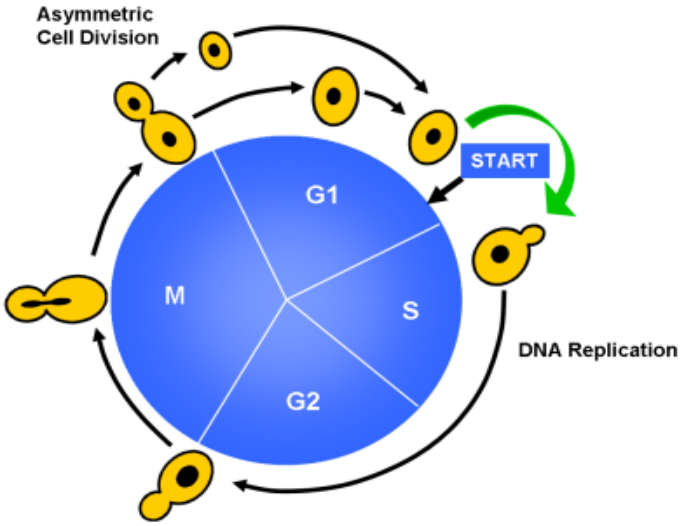
- The levels of Cln3 are sensitive to both cellular growth rate and metabolic state.
- Cln3-CDK1 phosphorylates and initiates the inactivation of the transcriptional inhibitor Whi5, promoting its disassociation from the transcription factor SBF (Swi4/Swi6).
- This results in weak transcriptional activation of two downstream G1 cyclins, *CLN1* and *CLN2*.
- Cln1 and Cln2 promote further inactivation of Whi5 and simultaneous activation of SBF and MBF (Mbp1/Swi6), which drive the cell cycle-dependent expression of over 200 genes including the S- phase cyclins that initiate DNA synthesis. The SBF component Swi4 is also an SBF target, suggesting an additional positive feedback loop.

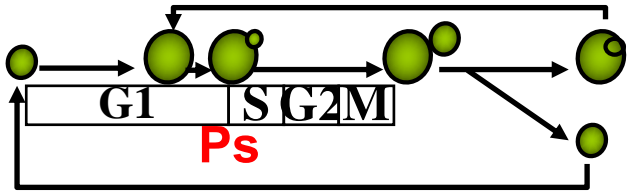
Mammalian cells



- The core G1 signaling network is similar in mammals, where growth factor stimulation leads to an increase in cyclin D, the upstream activator of G1 progression.
- Cyclin D, functionally analogous to the yeast Cln3 protein, activates CDKs 4 and 6 to phosphorylate and initiate inactivation of the pocket proteins p107, p130, and the retinoblastoma (Rb) protein.
- Inactivation of Rb leads to partial activation of the transcription factors E2F1-3, which then activate the transcription of downstream cyclins E and A that likely complete Rb inactivation and initiate DNA replication.

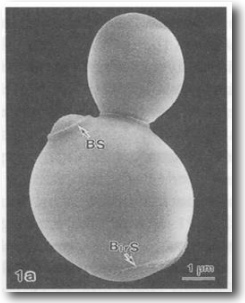
Cell cycle regulation in *Saccharomyces cerevisiae*

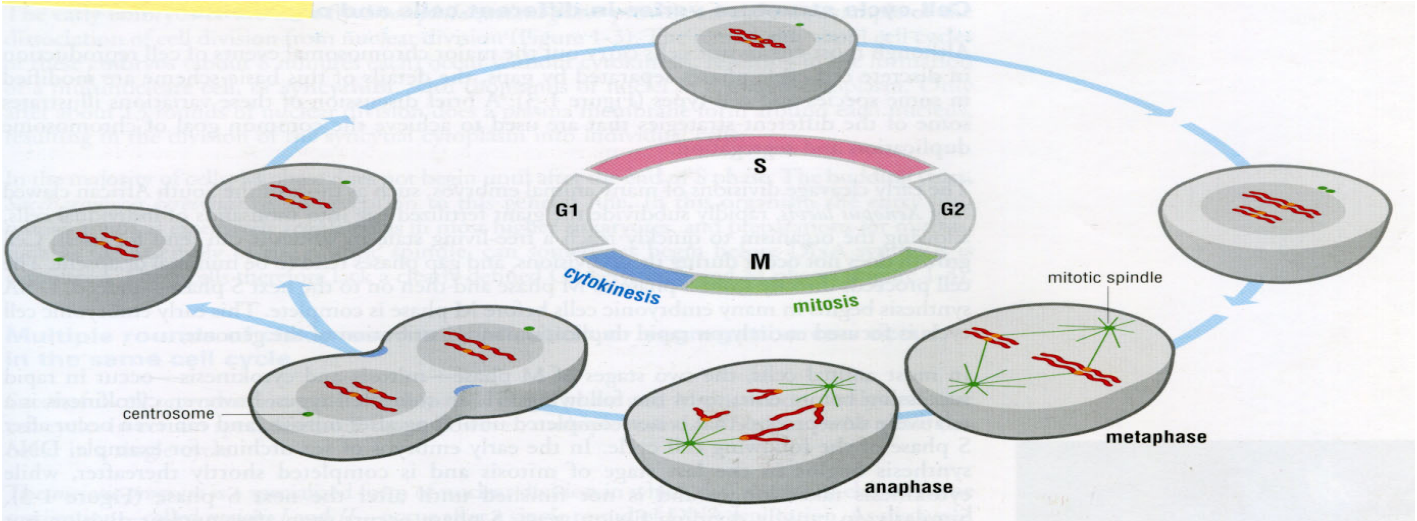




START:

- Budding
- DNA synthesis
- Spindle pole body duplication

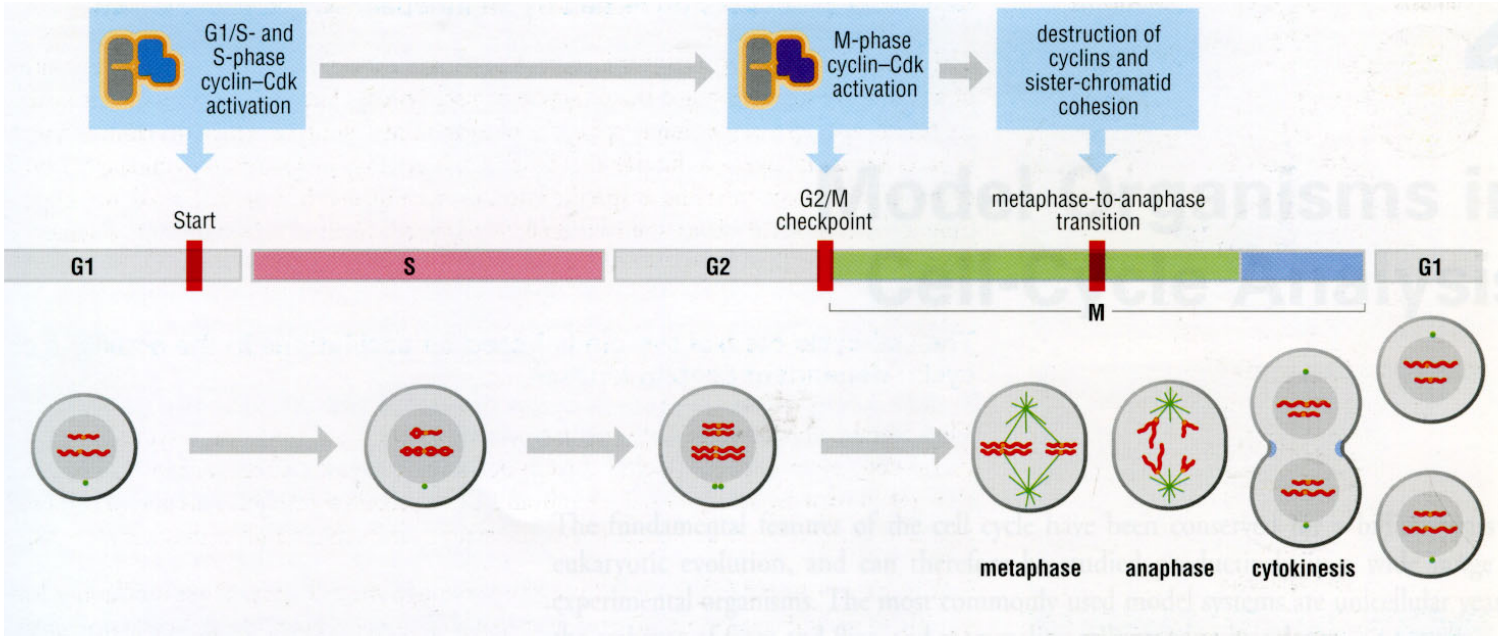




Cell cycle: the highly regulated series of events that leads to eukaryotic cell reproduction.

Cytoplasmic organelles, membrane, structural proteins and RNAs are replicated continuously though the cell cycle resulting in the gradual doubling of cell size at the end of the cycle; the centrosome is present in only one copy per cell, is duplicated strictly once per cell cycle, typically in S phase.

Cell-cycle control system



Cell cycle control system: a linked series of tightly regulated molecular switches, each of which triggers the initiation of cell-cycle events at a specific regulatory checkpoints

Cdk1 (Cyclin dependend kinase)
Cdc28 in *S. cerevisiae*

- Le molecole chiave che regolano le progressione attraverso le varie fasi del ciclo cellulare nelle cellule eucariotiche sono le chinasi ciclina dipendenti (CDK)

- *S. cerevisiae* possiede almeno 4 chinasi CDK: Cdc28, Pho85, Kin28, Srb10; tra queste solo la chinasi Cdc28 ha un ruolo preponderante nella regolazione del ciclo cellulare

- Isolato come mutante classe I di Start *(cdc28^{ts})* e clonato per complementazione funzionale
- Sequenza di *CDC28* presenta elevata omologia (62%) con la sequenza di *CDC2* di *S. pombe* (complementazione funzionale)

- *CDC28* è trascritto in modo costitutivo ed anche la proteina Cdc28

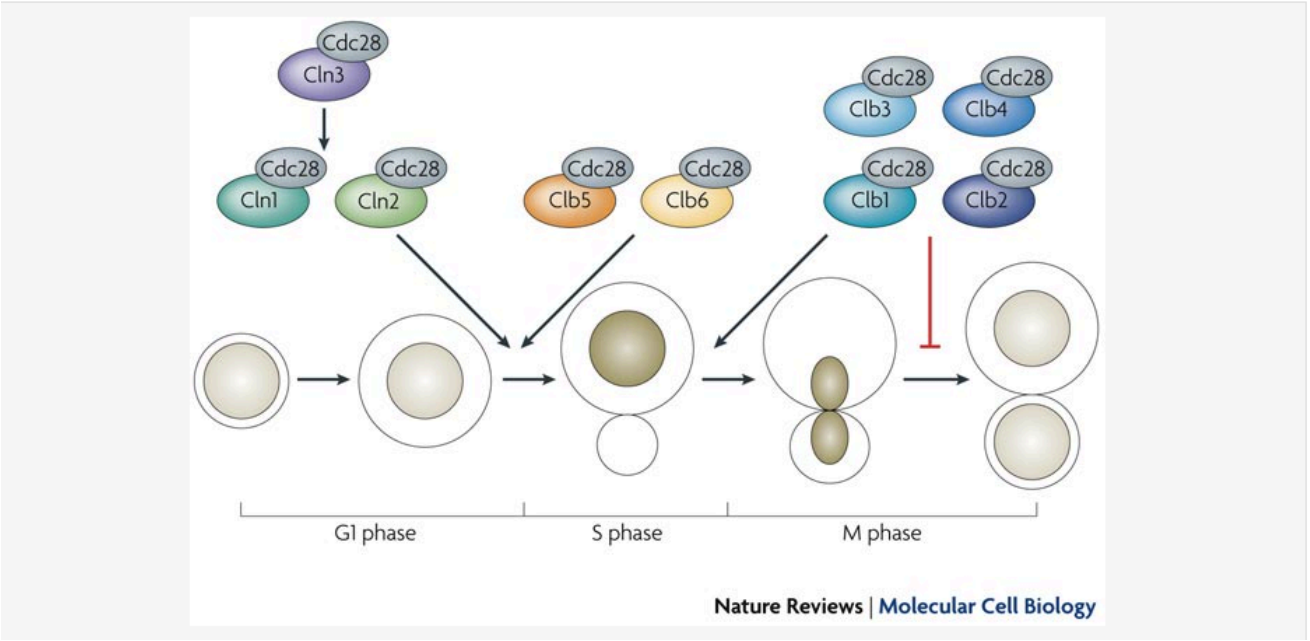
- *CDC28* codifica per una proteina di 34 KDa (p34) con attività chinasica su sequenze consenso: [S/T*]PX[K/R], where S/T* is the phosphorylated serine or threonine, P is proline, X is any amino acid, K is lysine, and R is arginine

- L'attività chinasica di p34 è regolata durante il ciclo

- Cellule arrestate in G1 per carenza nutrizionale o in presenza di ormoni coniugativi NON hanno attività chinasica associata a p34

- p34 purificata da cellule proliferanti è attiva e si trova associata a complessi di circa 200 Kda

Cdc28 in *S. cerevisiae*



- p34 è intrinsecamente inattiva
- L'attivazione dipende dall'associazione con subunità regolative, le CICLINE, la cui espressione è fase specifica
- L'attività dei complessi Cdc28/ciclina controlla le transizioni del ciclo cellulare
- Clns attivano la chinasi per le funzioni G1/S e le Clbs per le fasi S, G2 ed M

Joanna Bloom and Frederick R. Cross Multiple levels of cyclin specificity in cell-cycle control Nature Reviews Molecular Cell Biology 8, 149-160 (February 2007 doi:10.1038/nrm2105

Le cicline

- Le cicline sono proteine accumulate ciclicamente durante il ciclo cellulare
- Sono state identificate inizialmente in invertebrati marini come cicline mitotiche che presentavano un picco massimo all'inizio della mitosi per essere degradate alla fine e riaccumulate nell'interfase successiva
- Cicline mitotiche: cilcine A e cicline B. Hanno omologia di sequenza ma funzioni distinguibili, le cicline di tipo A non sono presenti in *S. cerevisiae*
- In *S. cerevisiae* sono state caratterizzate 11 cicline, di cui 9 si associano a Cdc28
- Cicline di fase G1: Cln3, Cln2, Cln1
- Cicline di fase S ed M: Clb5,6 e Clb1,2,3,4 (cicline di tipo B)
- Cicline Pcl1,2 si associano a Pho85

Le cicline

Le cicline di fase G1

Cln3: funziona a monte di ogni altro complesso ciclina/Cdk, è richiesta per l'espressione crescita dipendente delle altre cicline

Cln1,2: richieste per la gemmazione e la duplicazione del corpo polare del fuso e per il superamento di START

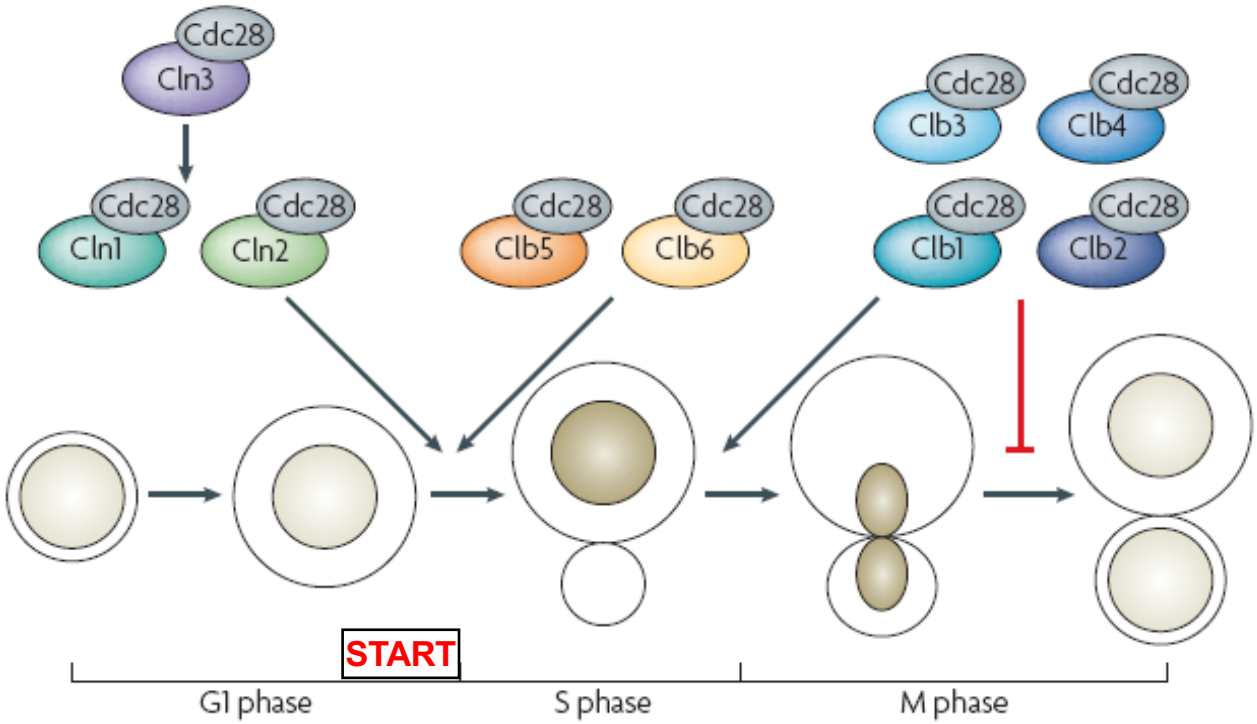
Le cicline di fase S

Clb5,6: cominciano ad essere sintetizzate in fase G1, presentano un picco in fase S e l'attività chinasica dei complessi Clb5,6-Cdk promuove la fase S

Le cicline di fase M

Clb1,2,3,4: sono richieste per la formazione ed il funzionamento dell'apparato mitotico

Cyclins in the budding yeast



Budding yeast cyclins activate a single cyclin-dependent kinase (Cdc28).
The **G1-phase** cyclins (Cln1, Cln2 and Cln3) promote bud emergence, spindle pole body duplication (not shown) and activation of the B-type cyclins.
The **S-phase** cyclins (Clb5, Clb6) advance DNA replication (shaded nucleus), and the **M-phase** cyclins (Clb1, Clb2, Clb3 and Clb4) promote spindle formation and the initiation of mitosis.
Mitotic cyclins inhibit mitotic exit and cell division.
Following cytokinesis, a mother and daughter cell are generated.

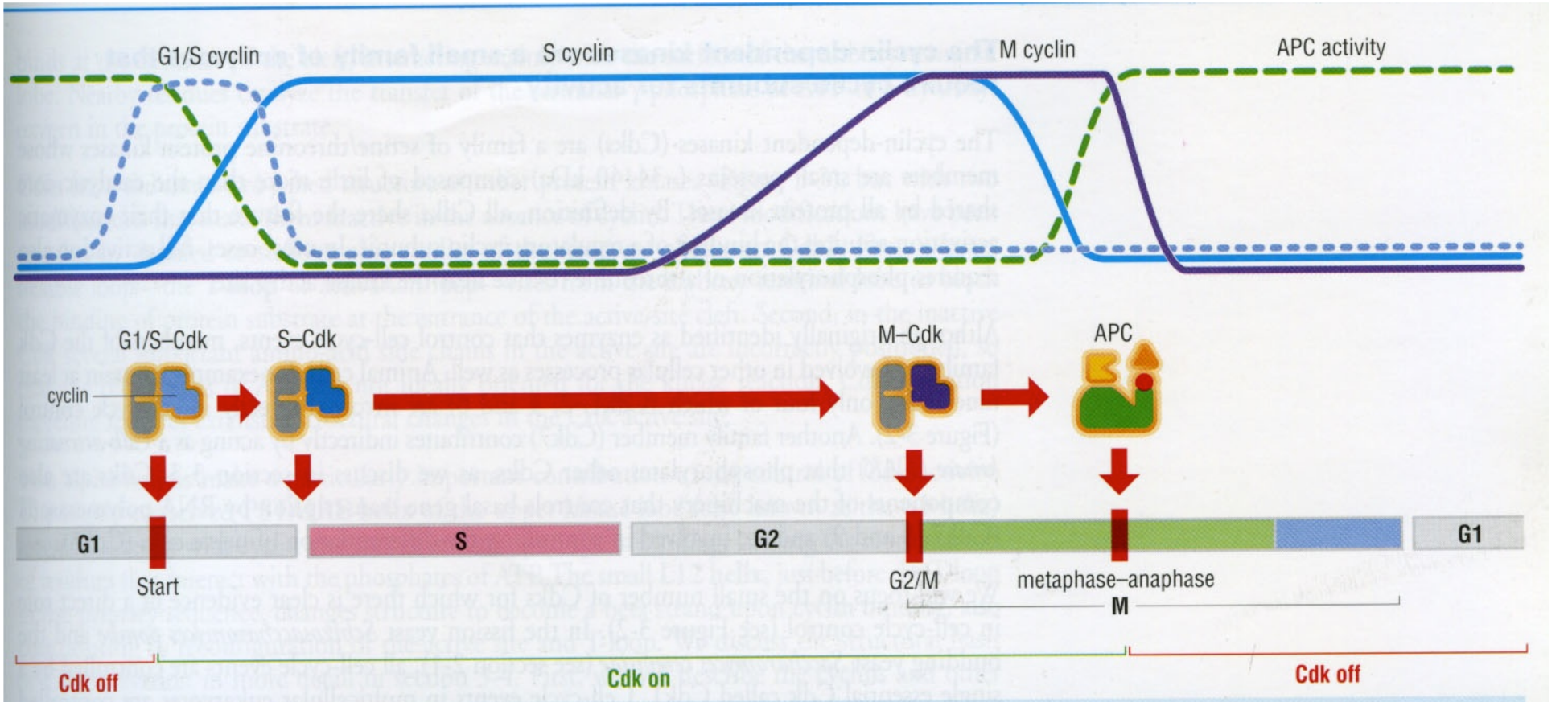
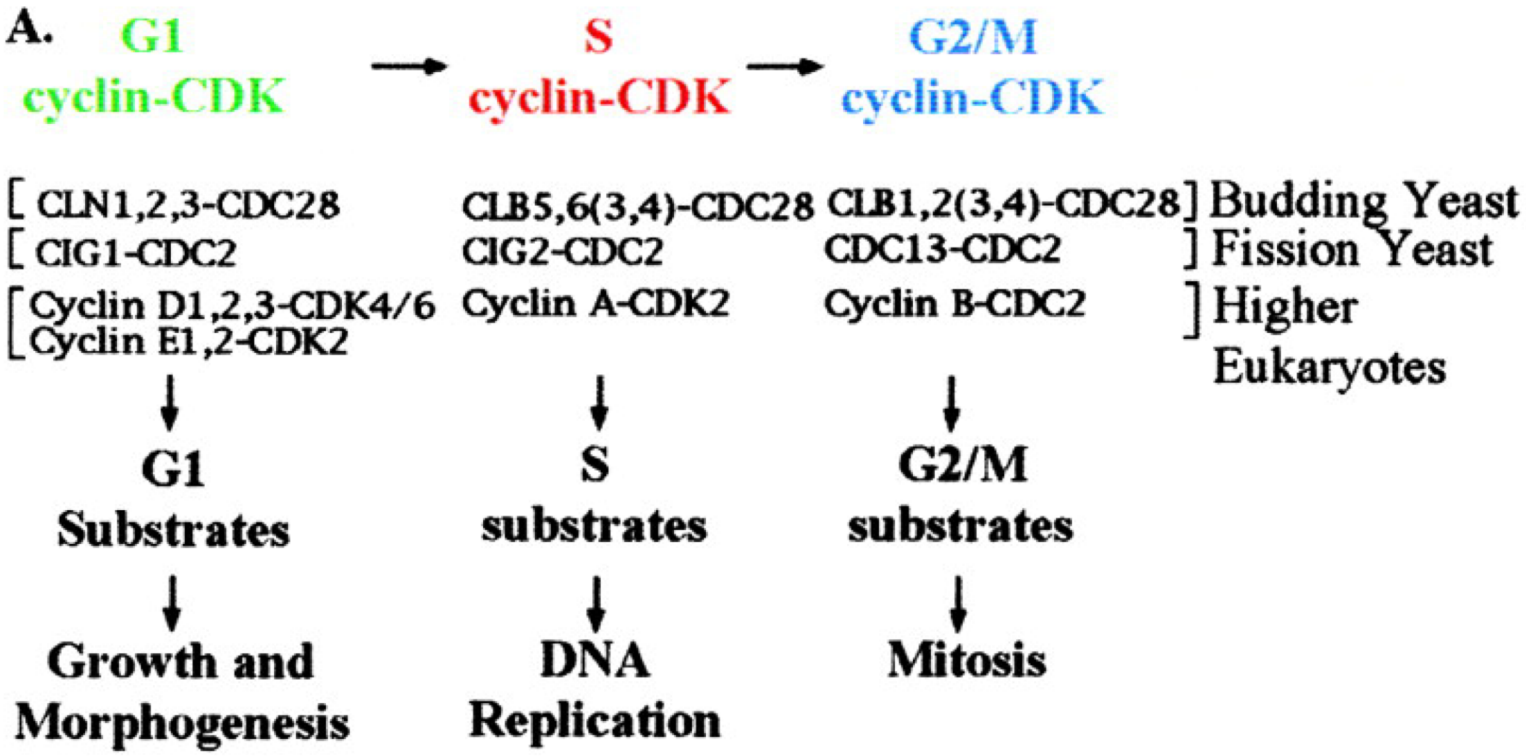
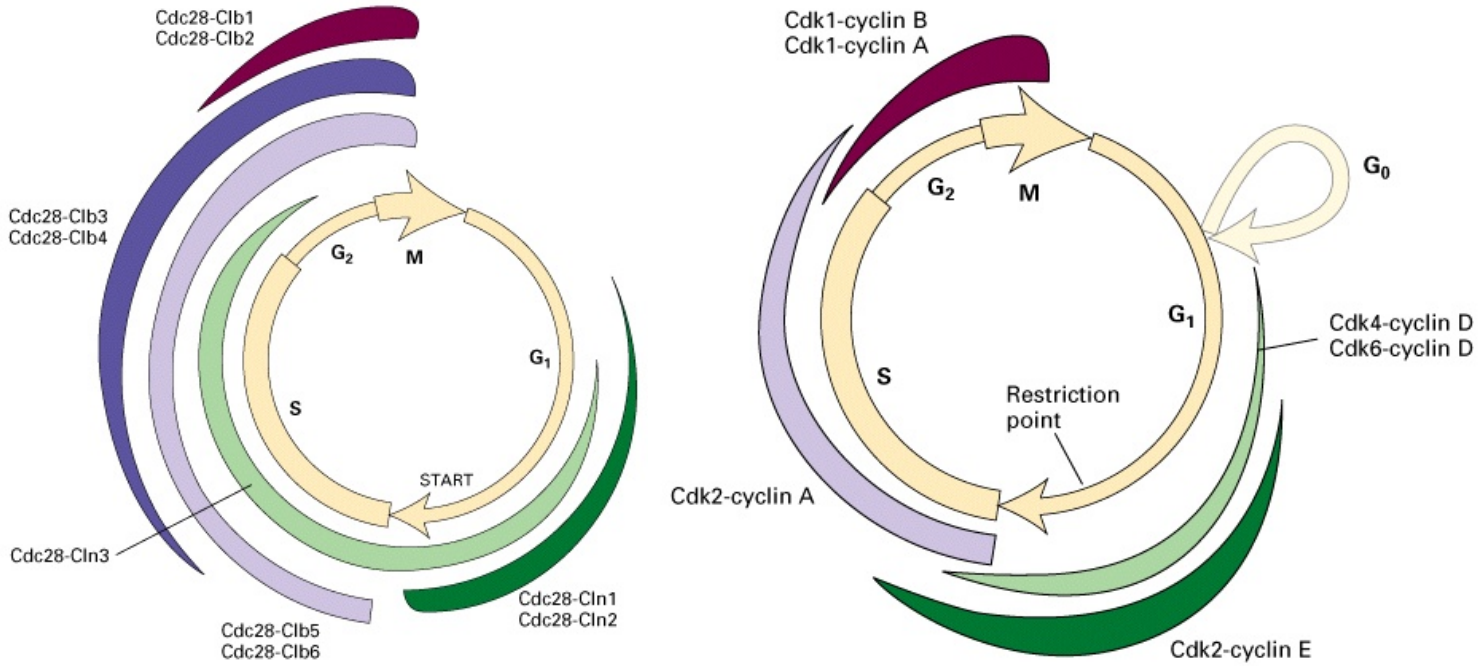


Figure 3-1 A simplified view of the cell-cycle control system Levels of the three major cyclin types oscillate during the cell cycle (top), providing the basis for oscillations in the cyclin–Cdk complexes that drive cell-cycle events (bottom). In general, Cdk levels are constant and in large excess over cyclin levels; thus, cyclin–Cdk complexes form in parallel with cyclin levels. The enzymatic activities of cyclin–Cdk complexes also tend to rise and fall in parallel with cyclin levels, although in some cases Cdk inhibitor proteins or phosphorylation introduce a delay between the formation and activation of cyclin–Cdk complexes. Formation of active G1/S–Cdk complexes commits the cell to a new division cycle at the Start checkpoint in late G1. G1/S–Cdks then activate the S–Cdk complexes that initiate DNA replication at the beginning of S phase. M–Cdk activation occurs after the completion of S phase, resulting in progression through the G2/M checkpoint and assembly of the mitotic spindle. APC activation then triggers sister-chromatid separation at the metaphase-to-anaphase transition. APC activity also causes the destruction of S and M cyclins and thus the inactivation of Cdks, which promotes the completion of mitosis and cytokinesis. APC activity is maintained in G1 until G1/S–Cdk activity rises again and commits the cell to the next cycle. This scheme serves only as a general guide and does not apply to all cell types.

Qualitative model for Cdk/Cyclin function



Restriction point/START in late G_1



Yeast Mammal

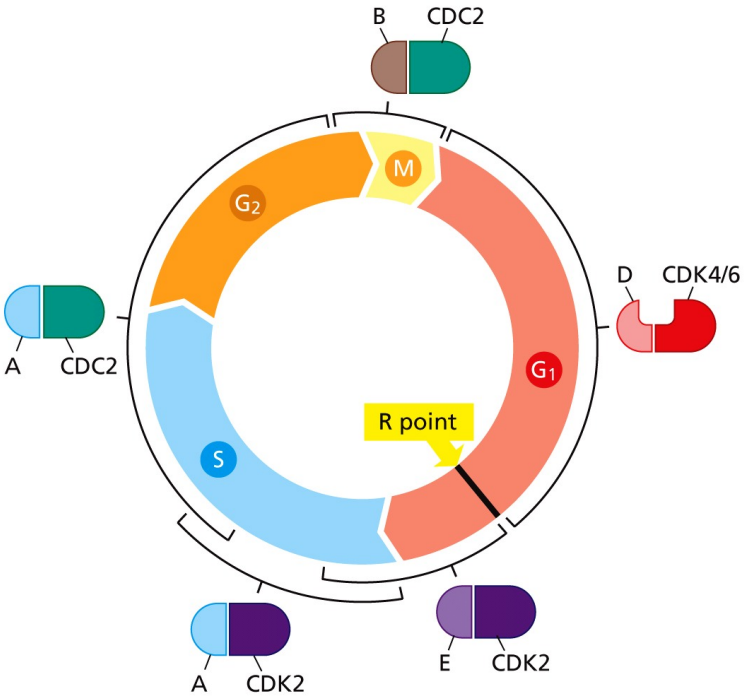


Figure 8.8 The Biology of Cancer (© Garland Science 2014)

- During the G1 phase CDK4 and CDK6 are associated with a trio of related cyclins (D1, D2 and D3), the D-type cyclins
- After the R point in late G1 the E-type cyclins (E1 and E2) associate with CDK2 to enable the phosphorylation of appropriate substrates required to entry into S phase
- As cells enter into S phase, the A-type cyclins (A1 and A2) replace E cyclins as the partners of CDK2 and thereby enable S phase to progress
- Later in S phase the A-type cyclins switch partners, leaving CDK2 and associating with CDC2 or CDK1
- As the cell moves further into G2 phase, the A-type cyclins are replaced as CDC2 partners by the B-type cyclins (B1 and B2) which trigger mitosis

Fluctuation of cyclin levels during the cell cycle

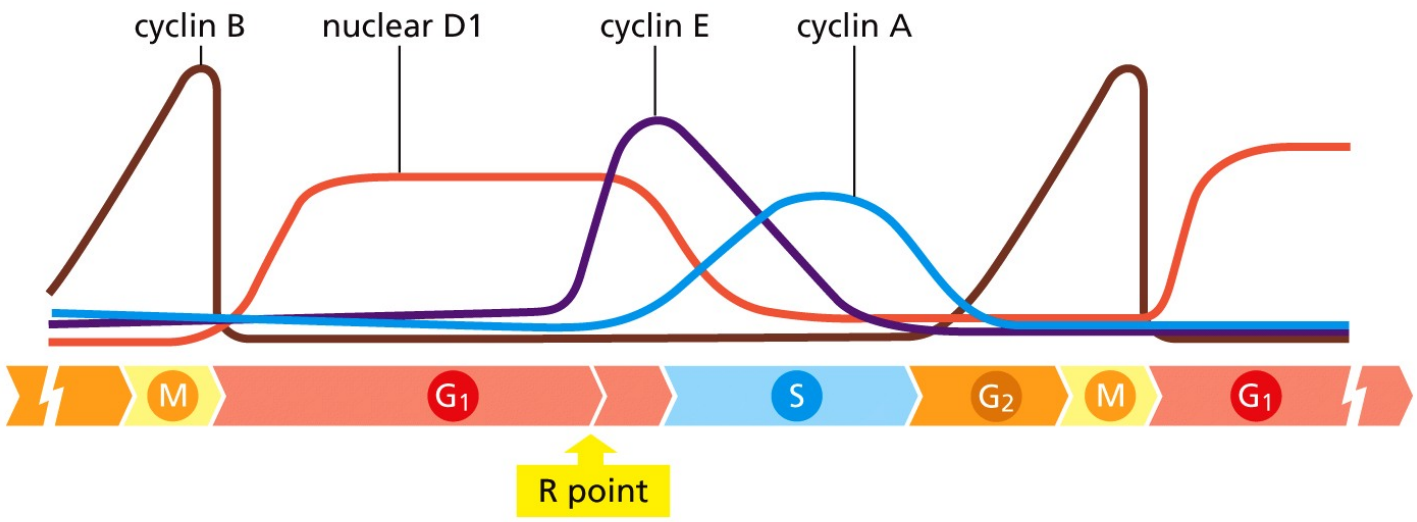


Figure 8.10 The Biology of Cancer (© Garland Science 2014)

The sole exception to these programmed fluctuations in the levels of cyclins is presented by the D-type cyclins. The levels of D1, D2 and D3 cyclins do not vary dramatically through the cell cycle

D-type cyclins are controlled by extracellular signals, mitogenic growth factors

Control of cyclin levels during the cell cycle

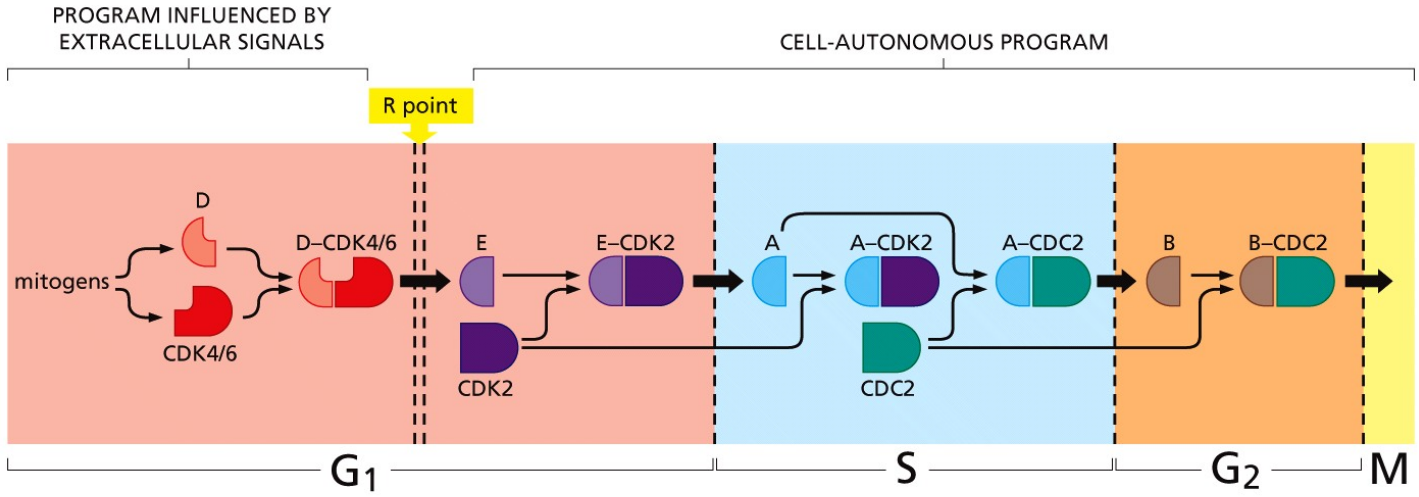


Figure 8.12 The Biology of Cancer (© Garland Science 2014)

- Extracellular signals strongly influence the levels of D-type cyclins during most of the G1 phase
- The levels of the other cyclins are controlled by intracellular signals