ONCOLOGY 520

"Cell Cycle Control"
January 31, 2012

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Concept 1
Cell cycle regulation: Cyclin dependent kinases

Concept 2
Mechanism of cell cycle regulation:
reversible phosphorylation
Cyclin dependent kinase inhibitors

Concept 3
Mechanism of Cell cycle regulation:
Ubiquitin mediated degradation drives the cell cycle

Concept 4
Dysregulation of the cell cycle in cancer

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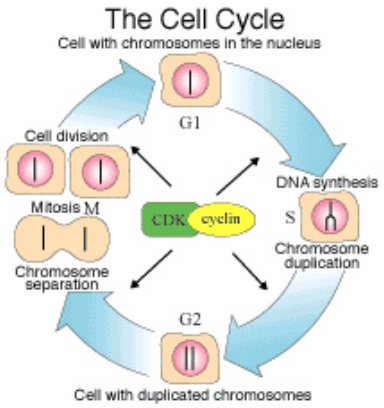
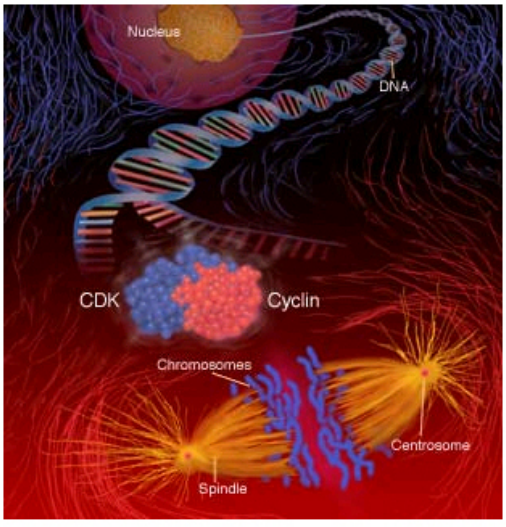


Organisms consist of cells that multiply through cell division. Before a cell can divide it has to grow in size, duplicate its chromosomes and separate the chromosomes for distribution between the two daughter cells. These different processes are coordinated in the cell cycle. The cell cycle consists of several phases. In the first phase (G1) the cell grows. When it has reached its appropriate size it enters the phase of DNA-synthesis (S), where the chromosomes are duplicated. During the next phase (G2) the cell prepares for division. In mitosis (M) the chromosomes separate, and the cell divides into two daughter cells. Through this mechanism the daughter cells receive identical sets of chromosomes. After division, the cells are back in G1 and the cell cycle is completed. The 2001 Nobel Laureates have discovered fundamental mechanisms controlling the cell cycle. CDK and cyclin drive the cell from one phase to the next in the cell cycle.

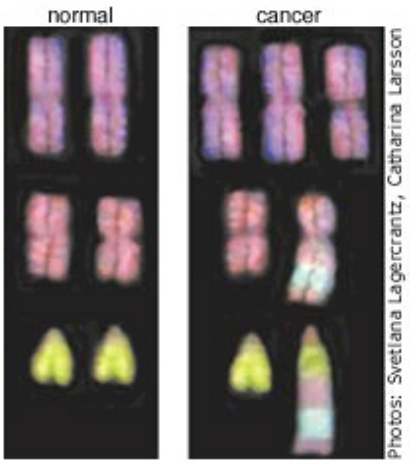
http://www.nobel.se/medicine/laureates/2001/illpres/index.html 3



The Nobel Prize in Physiology or Medicine 2001

The Nobel Assembly at Karolinska Institute has awarded the Nobel Prize in Physiology or Medicine jointly to **Leland Hartwell**, **Tim Hunt** and **Paul Nurse** for their discoveries of "key regulators of the cell cycle". Using genetic and biochemical methods, they identified the molecules CDK and cyclin that control the cell cycle in eukaryotic organisms. These fundamental discoveries have a profound impact on many aspects of biology and medicine. CDK and cyclin are key molecules that control and coordinate DNA synthesis, chromosome separation and cell division. CDK and cyclin together drive the cell from one cell cycle phase to the next.

http://www.nobel.se/medicine/laureates/2001/illpres/index.html 4



The Implications of the Discoveries

The basic discoveries made by this year's Laureates will have broad applications within many fields of biology and medicine. The discoveries are important in understanding how chromosomal instability develops in cancer cells, i.e. how parts of chromosomes are rearranged, lost or distributed unequally between daughter cells (figure to the left). The findings in the cell cycle field are about to be applied to tumour diagnostics, and the discoveries may in a long term perspective open new possibilities for cancer therapy. Chromosomal instability in cancer cells may be the result of defective cell cycle control. The figure shows three pairs of chromosomes (1, 3 and 14) in normal cells (left), compared with the same pairs in cancer cells (right). In cancer cells, the chromosome number may be altered (aneuploidy) and parts of chromosomes may be rearranged (visualized by different colours).

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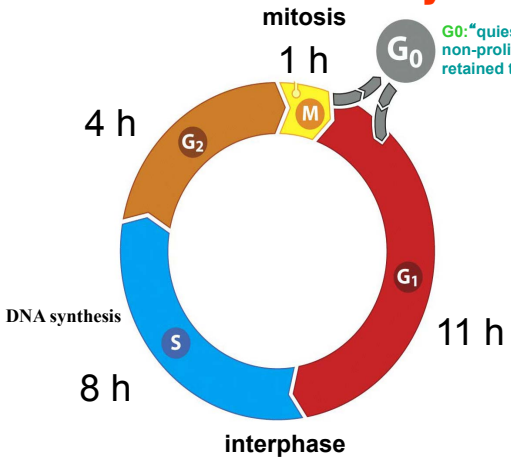
http://www.nobel.se/medicine/laureates/2001/illpres/index.html

The Cell Cycle



Average human cell cycle (Tc): 24 - 26 h *in vitro*
24 - 96 h *in vivo*

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Cell Growth: increase in mass

Cell Proliferation: cell division

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Entry into the Cell Cycle

The "growth cycle" and the "cell division cycle" are inextricably linked

Checkpoints and sensors monitor growth cycle

Growth factors

Metabolism & Organelle Biogenesis
(Glucose, amino acids, nutrients
Lipid and nucleotide biosynthesis)

pRb-E2F Induction of Cyclin Genes

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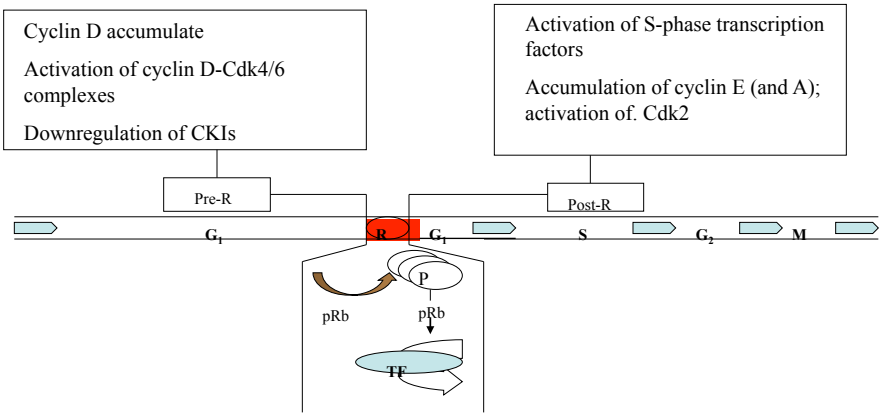
Pardee, 1993

R point (restriction point) - point in mid-to-late G_1 when the cell makes the decision to either progress through cell cycle or go to G_0 quiescent state

pRB protein is phosphorylated at R point



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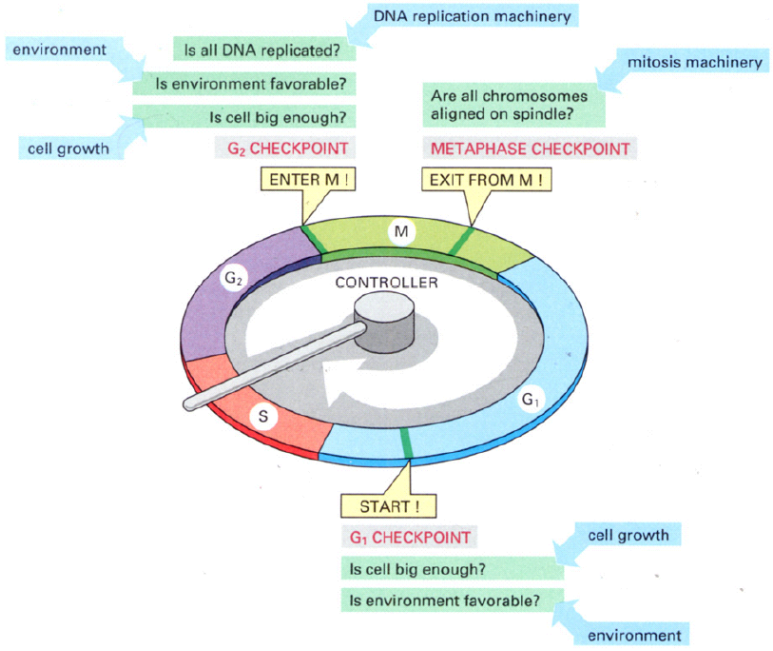

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Dean Jackson, Department of Biomolecular Sciences, University of Manchester Institute of Science and Technology

Cell Cycle

Coordination of the cell cycle results from changes in activity of cyclin dependent kinases (CDK).

Transitions in the cell cycle occur when activity of a given kinase activates proteins required for next phase of cell cycle.

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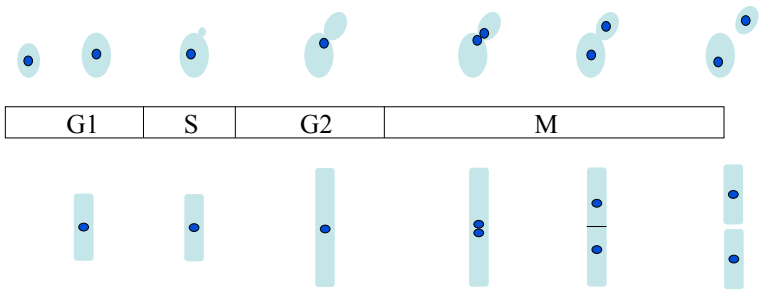
Yeast: a model for somatic cell cycle control

Budding yeast
Saccharomyces cerevisiae



Fission yeast
Schizosaccharomyces pombe

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Isolation of mutants defective in cell cycle control

a) Unable to proceed

b) Proceed with incorrect timing

Essential functions can be identified genetically by screening for conditional mutants: e.g. *temperature sensitive* mutants.

Temperature sensitive alleles generally encode gene products which are active at the **permissive temperature** but are inactive at the **restrictive temperature**.

A collection of *C*ell *D*ivision *Cycle* mutants was isolated and characterized independently in *S. cerevisiae* and *S. pombe*

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Hartwell, L.H. Review Genetics. Twenty five years of cell cycle genetics ***129, 975-980 1981***

Cell Cycle Terms

Cdc — cell division cycle

Products of these genes control the transit of cells at specific phases of the cell cycle

Cdk — Cyclin dependent kinase

serine/threonione protein kinase

inactive as monomers; active only after binding a cyclin partner

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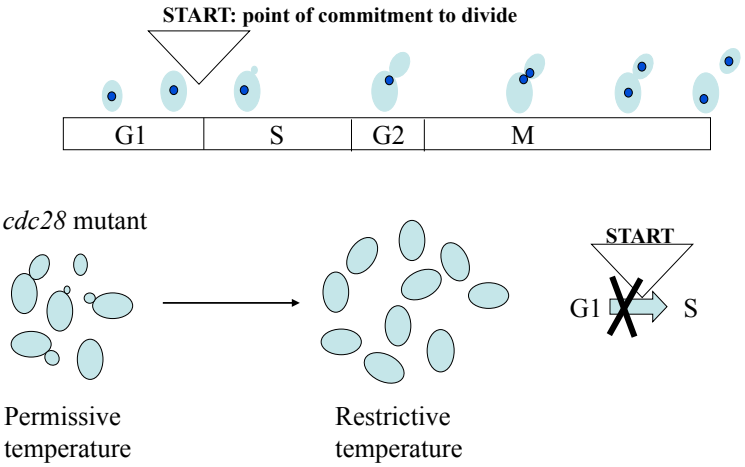
Mutation in *CDC28* results in failure to progress through START



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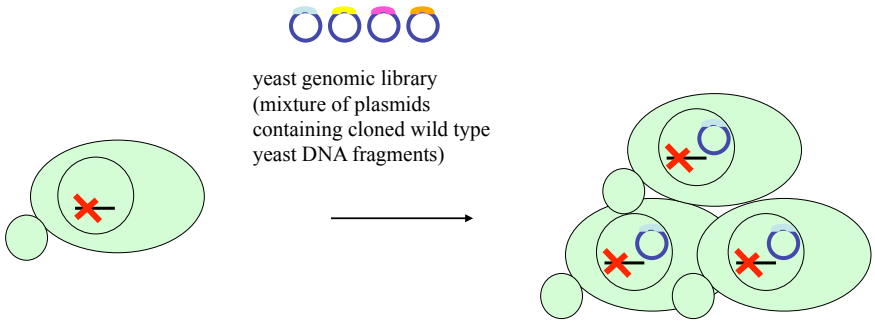
Isolation of a *cdc* gene by complementation



CDC28 was cloned. Sequence analysis predicted a 34kd protein with similarity to **kinases**. Ser/Thr Kinase activity was soon demonstrated.

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S. cerevisiae CDC28 corresponds to a *cdc* gene in *S. pombe, cdc2*$^{+}$

cdc2^{+} was identified in *S. pombe*: ***cdc2***$^{+}$ and ***CDC28*** are functional homologues (***CDC28*** can replace ***cdc2***$^{+}$ in *S. pombe* and vice versa)

By screening a human cDNA expression library in a *S. pombe* ***cdc2ts*** mutant, the corresponding human homologue was identified - and frogs, urchins and starfish

p34$^{cdc2/CDC28}$ is a **key cell cycle regulator** conserved throughout evolution

*Beach, D., Durkacz,B.., & Nurse, P. Functionally homologous cell cycle control genes in budding and fission yeast. Nature **300**, 706-709, 1982*

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...Purified MPF contained 32kd and 46 kd polypeptides

Maller and Lohka

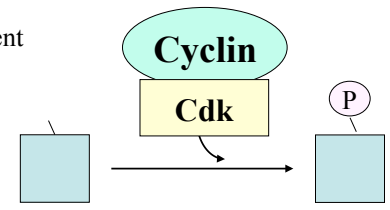
The 32kd component reacted with antibodies raised against cdc2+ protein

The 46kd component corresponded to a previously identified cyclin: Cyclin B

Cyclin dependent Kinase

Cyclin: regulatory component

Cdk: catalytic component



Lohka MJ, Hayes MK, Maller JL. 1988 Purification of maturation-promoting factor, an intracellular regulator of early mitotic events. Proc Natl Acad Sci U S A. 85(9): 3009-13.

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The CDK Family
Share high degree of structural homology
~ 34 kDa
Inactive as monomers

Cyclin Regulatory subunit
•Forms association with CDKs
•Accumulated during the cell cycle and are destroyed during mitosis

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Cyclin Regulatory subunit

Forms association with CDKs

Accumulated during the cell cycle and are destroyed during mitosis

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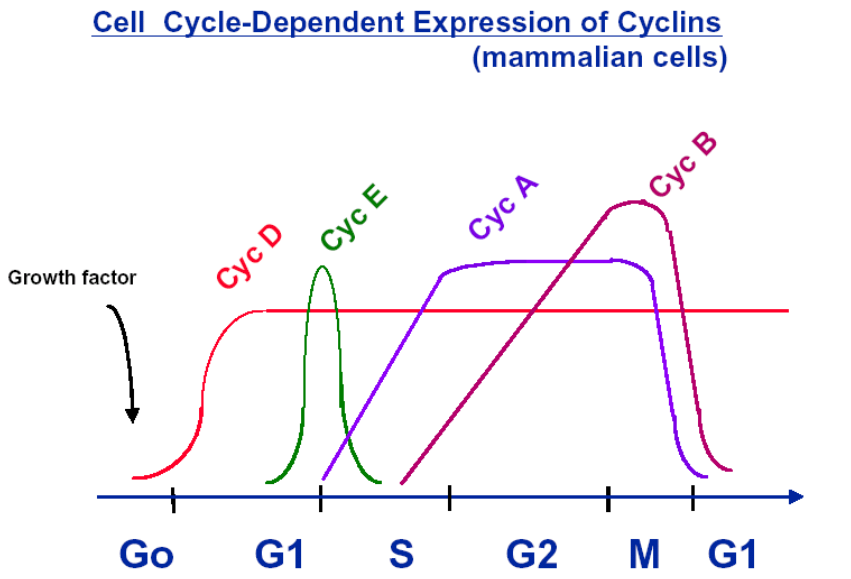

NOTE!!

The substrate specificity of the cyclin-Cdk complex depends on *both* the Cdk and the cyclin.

In non-proliferating cells, this flexibility allows the Cdks to perform roles separate from cell cycle regulation.

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Cell Cycle Motif

Regulation of the cell cycle by protein kinases that are activated by cyclins is a repeating principle.

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Multiple Cdks and inhibitors contribute to stage-specific regulation in metazoans

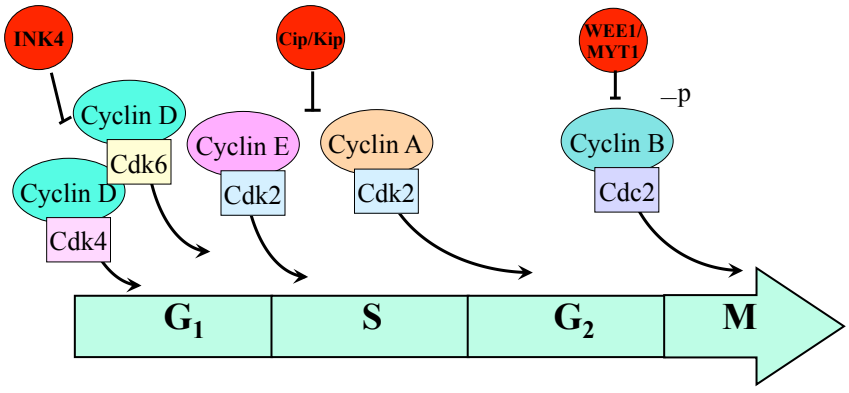

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Table 1. Cyclin-CDK complexes are activated at specific points of the cell cycle.

CDK	Cyclin partner	Cell cycle phase activity
CDK4	Cyclin D1, D2, D3	G1 phase
CDK6	Cyclin D1, D2, D3	G1 phase
CDK2	Cyclin E	G1/S phase transition
CDK2	Cyclin A	S phase and G2
CDK1 (cdc2)	Cyclin A	G2/M phase transition
CDK1 (cdc2)	Cyclin B	Mitosis
CDK7 (CAK, CDK activating kinase)	Cyclin H	CAK, all cell cycle phases
CDK5	p35	Neuronal differentiation
CDK8	Cyclin C	Transcription

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$G_o \rightarrow G1$

Cdk4 - Associates with cyclin D1 and D3

Cdk6 - Most closely related to cdk4
- Associated with cyclins D1, D2 and D3
  - CDK6/cyclin D phosphorylates RB
- Expressed in lymphocytes

Cyclin D - D1, D2, and D3
- Expression is partially cell type specific

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G1 → S

Cdk2
- Most closely related to cdc2
- Required - for onset of S
- Major partner is cyclin E

Cyclin E
- Associates with cdc2 and Cdk2
- Both cdc2/cyclin E and Cdk2/cyclin E have histone H1 kinase activity, but only Cdk2/cyclin E phosphorylates Rb

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S Phase

Cdk2
- Required - for onset of S
- S phase partner is cyclin A

Cyclin A
- Associates with cdk2
- Has a role in S phase progression (Cdk2-cyclin A complex is required for continued DNA replication)
- Co-localizes with components of the replication machinery

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G2 → M

Cdk1
- Same as cdc2
- Associates with cyclin B
- Required for G2 →M phase transition

Cyclin B
- Complexes with cdc2; promotes entry into mitosis
- Protein is first detectable during S phase but levels don't peak until G2/M

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The "Activating" Cdk

Cdk7
- The catalytic subunit of a protein kinase that can activate cdc2 and cdk2 kinases

- Together with **Cyclin H** forms CAK (cdk-activating kinase) which catalyzes phosphorylation of threonine 161 or corresponding sites on cdc2, cdk2, cdk4

- <u>Absolutely</u> required for the activity of these kinases

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The "Activating" Cyclin

Cyclin H
- Non-catalytic component of CAK
- Phosphorylates cdc2, cdk2, cdk4 complexed with cyclins but not those in the absence of cyclins
- Associates with TFIIH Phosphorylates CTD of RNA polymerase II
- Exact role in transcription remains to be determined

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Cyclin Grouping Based on Sequence Similarities

Group 1: Cyclins A, B, D1, D2, D3, E and F
Implicated in ***cell cycle control***

Group 2: Cyclins C and H
Potential role in ***transcriptional regulation***

Group 3: Cyclins F1, G2 and I
Mediating ***checkpoint*** in response to DNA damage

Group 4: p35
Lacks cyclin sequence similarity but functions as a ***CDK activator***

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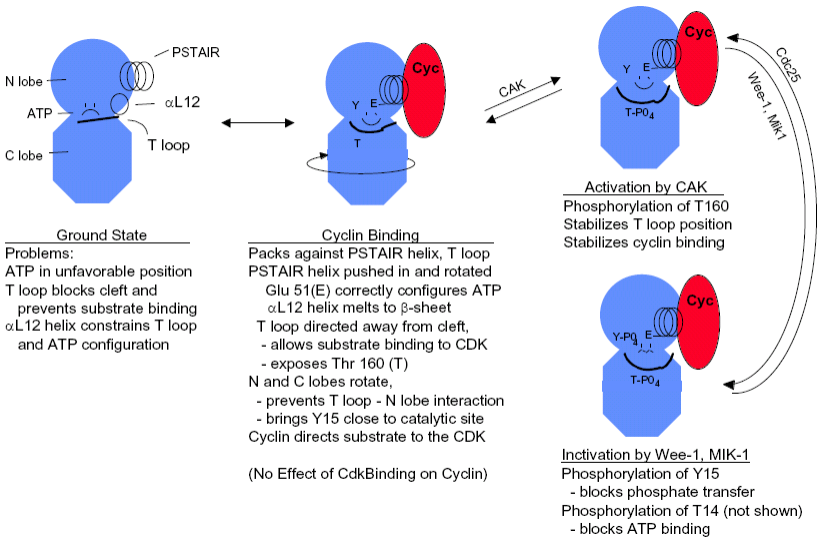
Oncogene 13:1103, 1996



Ground State
Problems:
ATP in unfavorable position
T loop blocks cleft and
prevents substrate binding
αL12 helix constrains T loop
and ATP configuration

Cyclin Binding
Packs against PSTAIR helix, T loop
PSTAIR helix pushed in and rotated
Glu 51(E) correctly configures ATP
αL12 helix melts to β-sheet
T loop directed away from cleft,
- allows substrate binding to CDK
- exposes Thr 160 (T)
N and C lobes rotate,
- prevents T loop - N lobe interaction
- brings Y15 close to catalytic site
Cyclin directs substrate to the CDK

(No Effect of CdkBinding on Cyclin)

Activation by CAK
Phosphorylation of T160
Stabilizes T loop position
Stabilizes cyclin binding

Inctivation by Wee-1, MIK-1
Phosphorylation of Y15
- blocks phosphate transfer
Phosphorylation of T14 (not shown)
- blocks ATP binding

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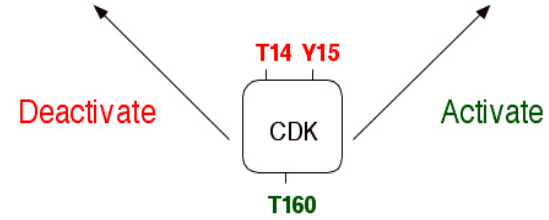
Regulating a CDK

Cyclin Proteolysis (Ubiquitin)	Cyclin Binding
Thr14 & Tyr15 Phosphorylation (wee1/mik1)	Thr14 & Tyr15 dephosphorylation (Cdc25)
T-loop Thr dephosphorylation ($p24^{KAP}$)	T-loop Thr phosphorylation (CAK)
Inhibitor binding	Inhibitor removal



Morgan 1995, Nature **374**, 131.

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Summary I:

Key concept – cell cycle is driven by Cdk/cyclin activity which is regulated by multiple phosphoryation/ dephosphorylation events

- The cell cycle is regulated by a family of cyclin dependent kinases (cdks) and its binding partner, cyclins.
- Cyclins are obligate positive binding partners of cdks.
- The cdk/cyclin complexes are further regulated by activating and inhibiting phosphorylation events.
- The T-loop contains the site of the activating phosphorylation and blocks the substrate binding site.
- Cdk activation is mediated in 2 steps:
  1. cyclin binding to Cdk induces conformational changes in the Cdk protein that alter the positions of catalytic residues and allows proper orientation of the ATP for catalysis (5 fold increase in activity). The T-loop is also displaced from the substrate cleft.
  2. Thr160 (Thr161 in Cdk1) is then phosphorylated by the Cdk-activating kinase (CAK), leading to a further 100-fold activation.

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Summary I *(cont.)*:

- Cdk activity is also regulated by inhibitory phosphorylations of Tyr15 and Thr14.
- The mechanism of inhibition is through blocking of ATP binding and phosphate transfer.
- Tyr15 and Thr14 are inaccessible in monomeric Cdks but become exposed when cyclin is bound to Cdk.
- Wee1 and Myt1 kinases are responsible for the inhibitory phosphorylation.
- The Cdc25 phosphatase is responsible for removing the inhibitory phosphorylation.
- The multiple levels of control allows for precise regulation.

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Cdk Inhibitors (CKIs)

KIP Family (Kinase Inhibitory Protein)

p21
p27
p57

INK4 Family (Inhibitor of CDK4)

p16
p14
p15
p18

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p21 aka Cip1, WAF1

Transcription is p53 dependent

Inhibits virtually all Cdks

Contains independent Cdk and PCNA binding domains each of which is required and sufficient for inhibition of these proteins

Expression elevated in terminally differentiated cells

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p27 aka Kip1

Shares sequence homology with p21

Is induced by TGF-β and cell-cell contact

Expression elevated in quiescent cells

Both p21 and p27 block Cdk/cyclin dimers from being a substrate for activation by CAK

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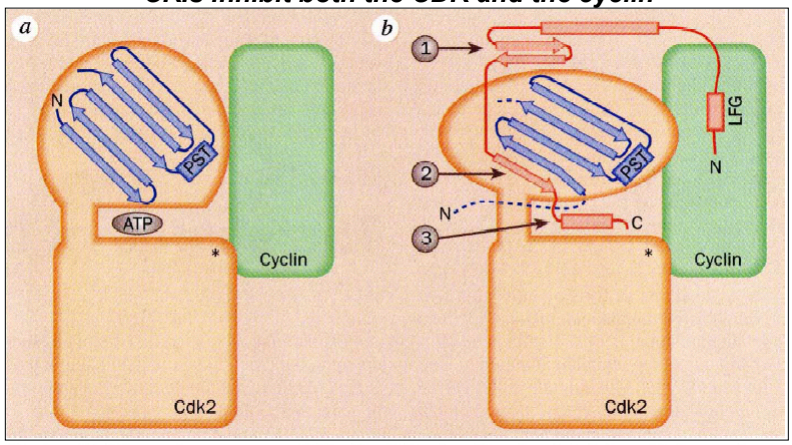
CKIs inhibit both the CDK and the cyclin



The effects of p27 on the structure of the Thr-160-phosphorylated Cdk2–cyclin A complex. *a*, A highly simplified view of the active, Thr-160-phosphorylated complex[9]. Cdk2 (yellow) contains two lobes separated by the active-site cleft; the upper amino-terminal lobe contains a five-stranded β-sheet (blue) and the PSTAIR helix ('PST'). Phosphorylated Thr 160 (labelled with a large asterisk) is found in the T-loop at the base of the cleft, where protein substrate presumably binds. The p27-bound complex[1] is shown in *b* (p27 is in red). The LFG motif near the amino terminus of the p27 peptide binds cyclin. In the carboxy-terminal half of the p27 peptide, the three substructures that interact with Cdk2 are numbered as follows. (1) The β-turn of p27 forms a sandwich with the Cdk2 β-sheet. (2) The β-strand of p27 displaces the first strand of the Cdk2 sheet, which is now disordered (dashed line). (3) The p27 3_{10} helix occupies the ATP-binding site. Morgan *Nature* 382:295-296, 1996

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p57 aka Kip2

Sequence similarity to p27 but not p21

Has p21/p27 inhibitory domain

Expressed in *tissue specific manner* (placenta, muscle, heart) suggesting a specialized role in cell cycle control

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INK4 Family (Inhibitor of CDK4)

Associates with and inhibits Cdk4 and Cdk6

Family members are structurally similar

No similarity to Cip/Kip proteins

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p16 aka MTS1, INK4a

Inhibitor of Cdk4 and Cdk6

$p14^{ARF}$ Alternate reading frame protein
$p19^{ARF}$ encoded by the p16 locus

No amino acid similarity to p16 or other proteins

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p15 aka INK4b

Adjacent to *INK4* locus

Frequently co-deleted with p16

Up-regulated by TGF-β in cascade effect (displaces p27 which is then free to bind cyclin E/Cdk2 to result in G1 arrest.)

p18 Predominately expressed in hematopoietic (blood) cells

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Structural Effects of Cip/Kips and INKs

p27 Binds CyclinA/Cdk2

Binds both cyclin and Cdk

N-terminus binds cyclin groove to block substrate interaction

C-terminus destabilizes ATP binding

C-terminus binds T-loop, blocks CAK

Sequence conservation suggests similar mechanism for p21 and p57

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p19 Binds Cdk6

Binds opposite face from cyclin

Binds catalytic cleft, distorts ATP binding site

Attracts T-loop, prevents substrate entry and Thr160 phosphorylation

Contacts residues specific to Cdk4/6 (these residue are *not* find in Cdk1 or Cdk2)

Sequence conservation suggests similar mechanism for p15, p16 and p18

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Summary II:

Key concept – Cdk/cyclin kinase activity is regulated at multiple levels

- Two families of CKI inhibitors, CIP/KIP and INK4.
  1. The CIP/KIP (p21 and p27) proteins bind to Cdk/cyclin and block block substrate interaction, destabilize ATP binding and block T-loop CAK phosphorylation.
  2. INK4 family proteins inhibit binding of Cdk4 and 6 to D-type cyclins
  3. INK4 proteins can also inhibit the activity of preassembled cyclin D/cdk4 and cyclin D/cdk6 complexes.

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Summary II (cont.): Levels of Regulation

- Each cyclin protein is synthesized at a discrete stage.
- Cyclin degradation is regulated.
- Cyclin/CDK complexes are activated by regulated kinase activity.
- Deactivation of CDK activity by phosphorylation of ATP binding site or reactivation by phosphatases.
- CDK inhibitors block assembly of the complex or activation of kinase activity.

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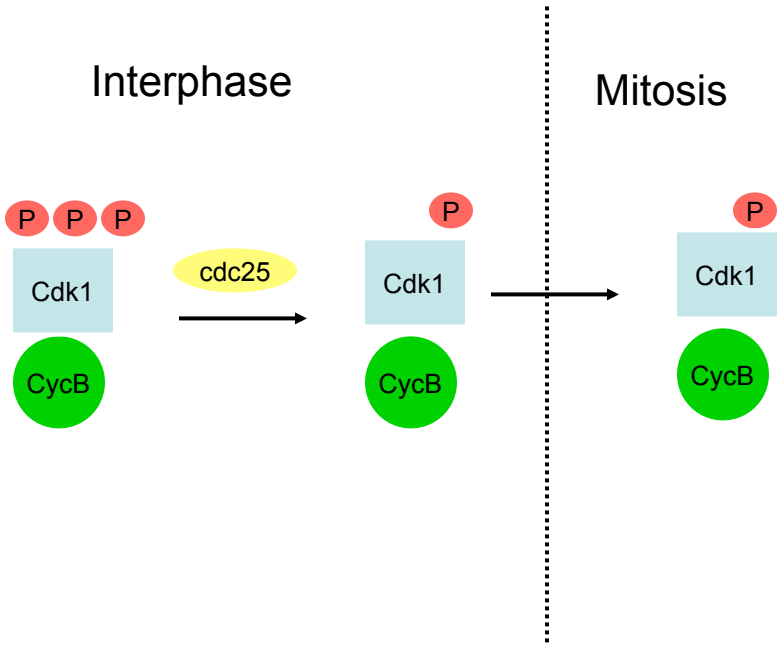

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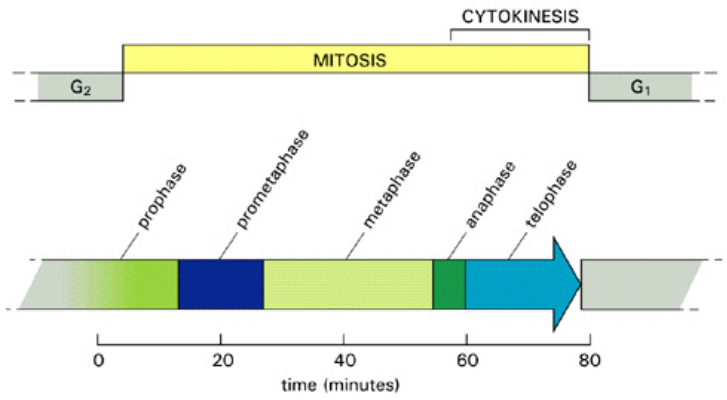

Figure 18-7. A typical time course for mitosis and cytokinesis (M phase) in a mammalian cell. The times vary for different cell types and are much shorter in embryonic cell cycles. Note that cytokinesis begins before mitosis ends. The beginning of prophase (and therefore of M phase as a whole) is defined as the point in the cell cycle at which condensed chromosomes first become visible - a somewhat arbitrary criterion, since the extent of chromosome condensation appears to increase continuously during late G2. The beginning of prometaphase is defined as the time when the nuclear envelope breaks down.

Molecular Biology of the Cell, 3rd edn. Part III. Internal Organization of the Cell Chapter 18. The Mechanics of Cell Division

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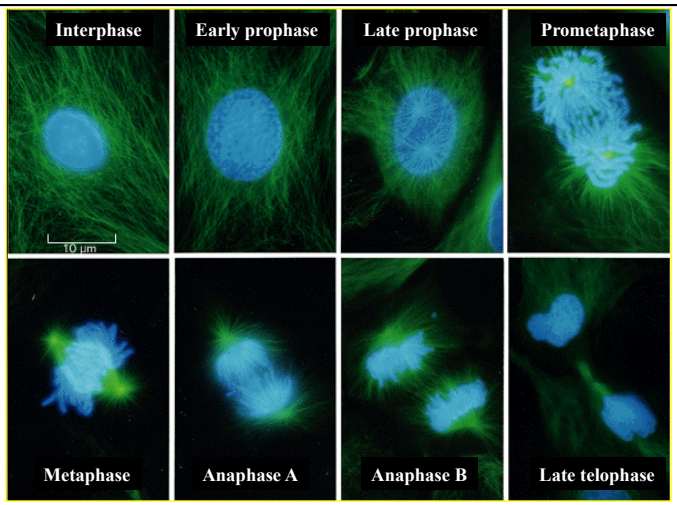

Mitosis in a typical animal cell. In these micrographs of cultured newt lung cells, the microtubules is green, while chromatin is stained with a blue fluorescent dye. During *interphase* the centrosome, consisting of matrix associated with a centriole pair, forms the focus for the interphase microtubule array. By *early prophase* the single centrosome contains two centriole pairs (not visible); at *late prophase* the centrosome divides and the resulting two asters move apart. The nuclear envelope breaks down at *prometaphase*, allowing the spindle microtubules to interact with the chromosomes. At *metaphase* the bipolar spindle structure is clear and all the chromosomes are aligned across the middle of the spindle. The paired daughter chromosomes, called *chromatids*, all separate synchronously at *early anaphase* and, under the influence of the microtubules, begin to move toward the poles. By *late anaphase* the spindle poles have moved farther apart, increasing the separation of the two groups of chromosomes. At *telophase* the daughter nuclei re-form, and by *late telophase* cytokinesis is almost complete, with the midbody persisting between the daughter cells. (Photographs courtesy of C.L. Rieder, J.C. Waters, and R.W. Cole.)

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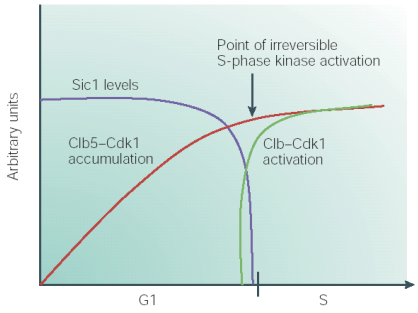

Figure 2 | **Creating an irreversible cell-cycle transition.**
The gradual kinetics of Clb5–Cdk1 accumulation are shown alongside the rapid kinetics of Clb5–Cdk1 activation in the budding yeast, *Saccharomyces cerevisiae*. The ability to accumulate inactive Clb5–Cdk1 molecules is mediated by the Cdk inhibitor Sic1. The concerted destruction of Sic1 — by G1-cyclin–Cdk-complex-mediated phosphorylation and Cdc4-mediated ubiquitylation — and concomitant activation of Clb5–Cdk1, promotes an irreversible transition to S phase. G1, Gap phase 1; S, DNA synthesis.

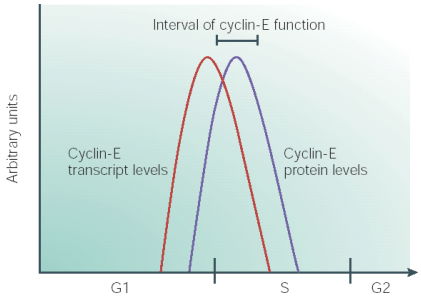

Figure 3 | **Limiting the interval of expression to the interval of required function.**
Periodic transcription of cyclin E at the G1–S boundary coupled with ubiquitin-mediated proteolysis of active cyclin-E–Cdk2 complexes limits the interval of cyclin-E protein accumulation in mammalian cells. Cyclin-E autophosphorylation is followed by Cdc4-mediated ubiquitylation and degradation of cyclin E. Evidence indicates that persistence of cyclin E outside of this window can be deleterious to cells. G1 and G2, Gap phases 1 and 2; M, Mitosis; S, DNA synthesis.

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"The cell cycle is a series of degrading events"

Cell cycle is regulated by destruction of cyclins

Ubiquitin-mediated proteolysis

A "destruction box" is shared by all mitotic cyclins

There may be cyclin-specific ubiquitin-conjugating enzymes that may be active only at certain times of the cell cycle.

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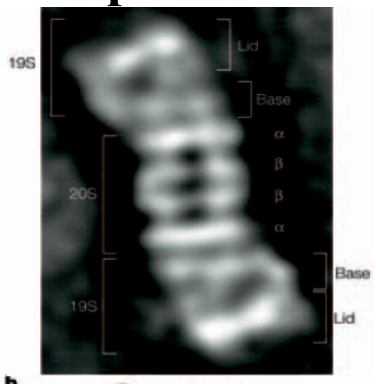
Ubiquitin

26S proteasome



Ciechanover, A. (2005). From the lysosome to ubiquitin and the proteasome. Nature Rev. Mol. Cell Biol. 6, 79–86.

54

Nobel Prize for Chemistry 2004

Proteins that are marked for hacking into small pieces

It has long been clear how proteins are built up in the cell. But the opposite, how they are broken down, was long thought to be less exciting to study. This year's Nobel Laureates, Aaron Ciechanover, Avram Hershko and Irwin Rose, went against the stream and, at the beginning of the 1980s, discovered one of the cell's most important control mechanisms, controlled protein degradation.

Irwin Rose College of Medicine, University of California, Irvine, USA

Avram Hershko Rappaport Institute, Technion – Israel Institute of Technology Haifa, Israel

Aaron Ciechanover Rappaport Institute, Technion – Israel Institute of Technology Haifa, Israel

The discovery was made at the beginning of the 1980s at the Fox Chase Cancer Center in Philadelphia, USA, jointly by the three scientists.

55

Vol. 81, No. 4, 1978
April 28, 1978

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 1100-1105

A HEAT-STABLE POLYPEPTIDE COMPONENT OF AN ATP-DEPENDENT PROTEOLYTIC SYSTEM FROM RETICULOCYTES

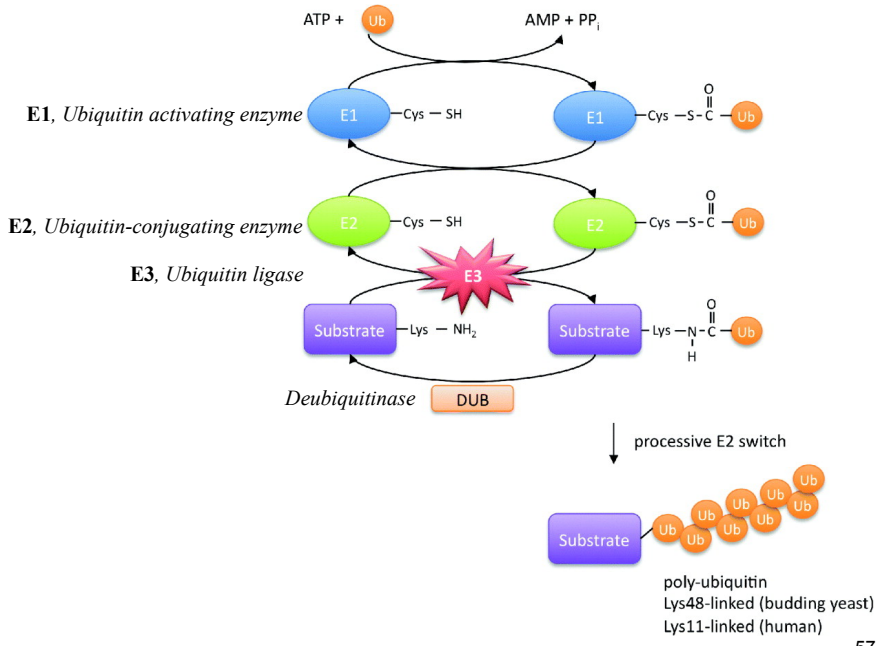
Aharon Ciehanover, Yaacov Hod and Avram Hershko[1]

Technion-Israel Institute of Technology, School of Medicine, Haifa, Israel

Received March 8,1978

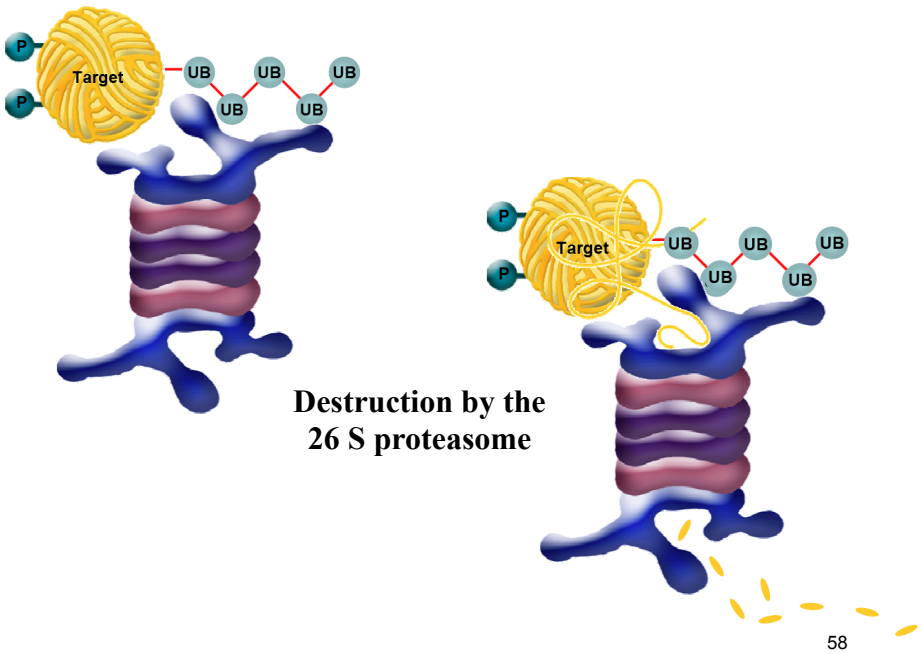
SUMMARY: The degradation of denatured globin in reticulocyte lysates is markedly stimulated by ATP. This system has now been resolved into two components, designated fractions I and II, in the order of their elution from DEAE-cellulose. Fraction II has a neutral protease activity but is stimulated only slightly by ATP, whereas fraction I has no proteolytic activity but restores ATP-dependent proteolysis when combined with fraction II. The active principle of fraction I is remarkably heat-stable, but it is non-dialysable, precipitable with ammonium sulfate and it is destroyed by treatment with proteolytic enzymes. In gel fil-

56



Modified from Passmore & Barford (2004)

57

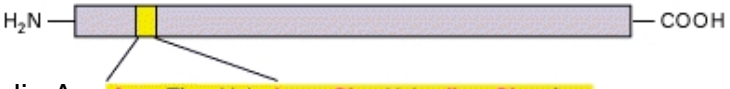


58

Mitotic cyclins have destruction box

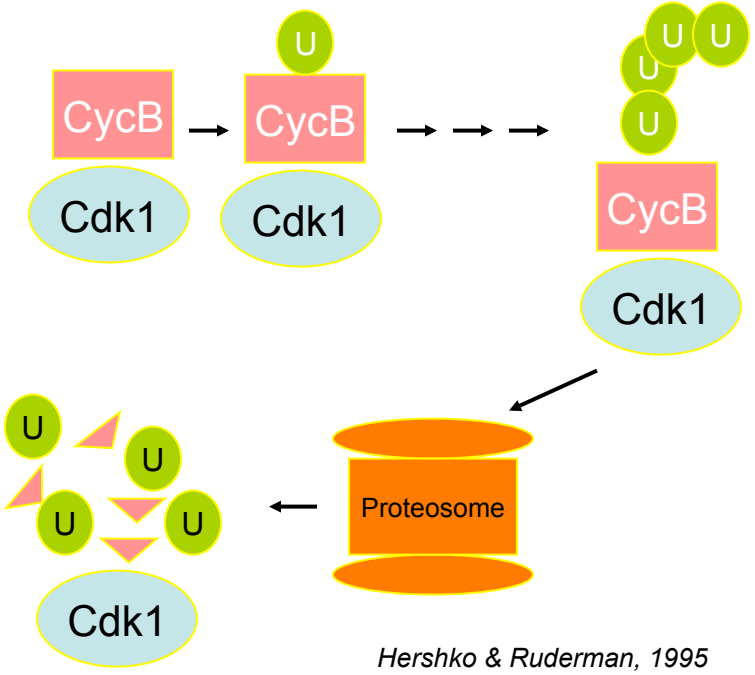
- Mitotic cyclins have a 9 residue "destruction box
- This is recognized by ubiquitinating enzymes that mark a protein for degradation.
- This process is directed by APC (anaphase promoting complex)

Mitotic cyclin destruction box



Cyclin A	Arg–Thr–Val–Leu–Gly–Val–Ile–Gly–Asp
Cyclin B1	Arg–Thr–Ala–Leu–Gly–Asp–Ile–Gly–Asn
Cyclin B2	Arg–Ala–Ala–Leu–Gly–Glu–Ile–Gly–Asn

59



Hershko & Ruderman, 1995

60

States of the Cell Cycle are generated by Proteolysis

Different complements of proteins are present in different cell cycle states

61

The Cell Cycle is Co-ordinated by Ubiquitin-dependent Proteolysis

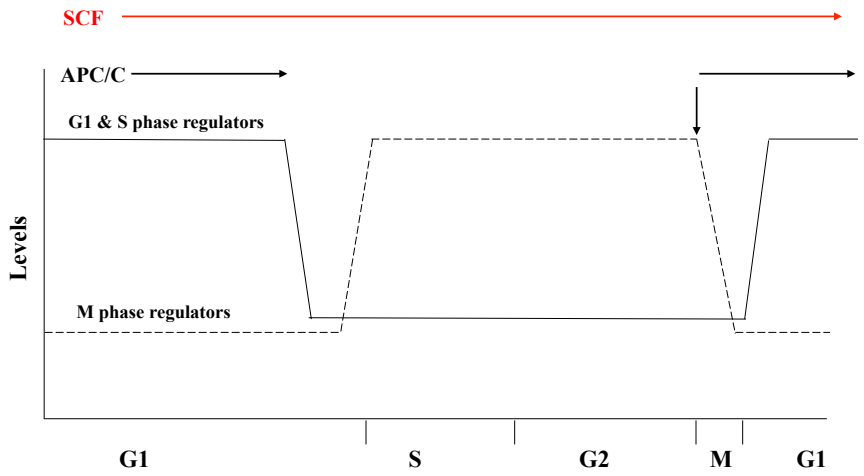
Effectively an interplay between the SCF and the APC/C

SCF = Skp1 + Cullin + F-box protein

APC/C = Anaphae Promoting Complex/Cyclosome

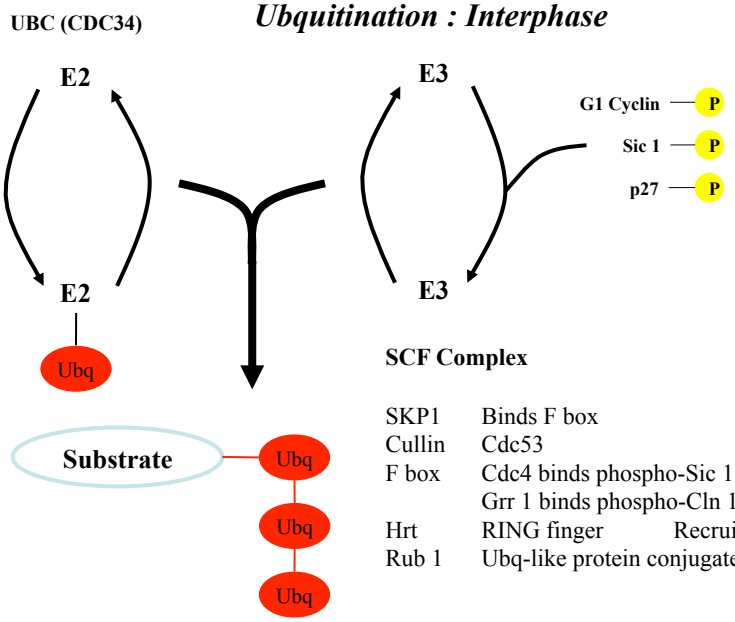
62

Proteolysis underlies the alteration of the cell cycle phases



63

Ubquitination : Interphase



SCF Complex

SKP1	Binds F box
Cullin	Cdc53
F box	Cdc4 binds phospho-Sic 1 and Far 1 Grr 1 binds phospho-Cln 1
Hrt	RING finger Recruits UBC
Rub 1	Ubq-like protein conjugated to cullin

64

Zachariae and Nasmyth 1999, Genes Dev 13, 2039.

Structure of the SCF ligase complex

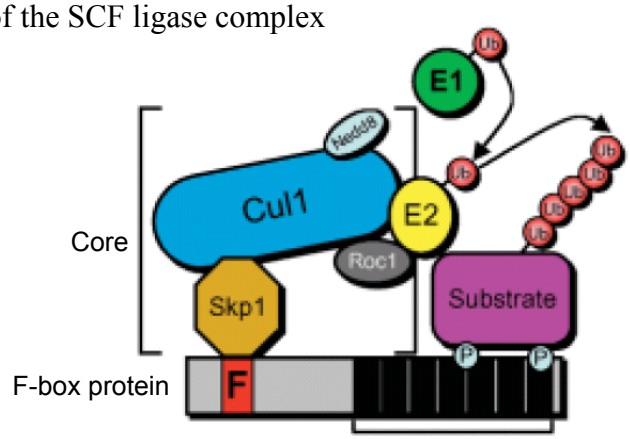
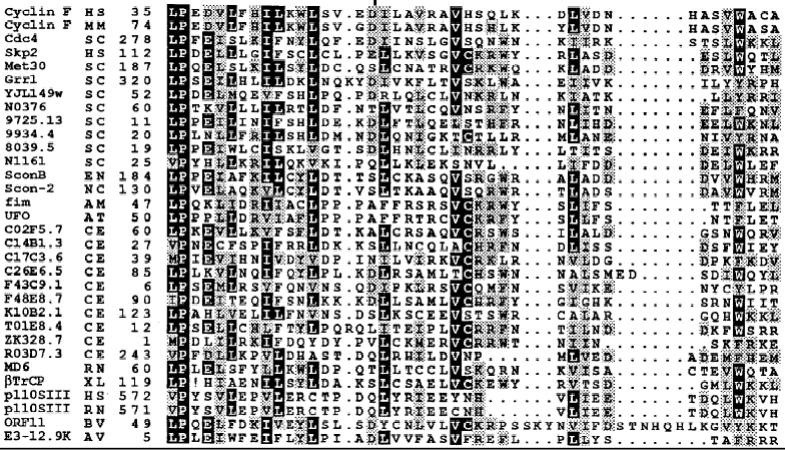


Figure 2. Architectural structure of the SCF ligase complex. Cul1, Skp1, and Roc1 form the invariant core of the SCF ligase. F-box proteins bind specific substrates, which are usually phosphorylated, through their C-terminal binding domains (such as WD40 and LRR repeats) and recruit them to the SCF core through interaction of their amino-terminal F-box motif with Skp1. Cul1, which is modified through covalent attachment of Nedd8, acts as a rigid scaffold to mediate the interaction between the substrate and the E2. The RING finger protein Roc1 is an essential component of SCF that stimulates ubiquitination activity in vitro.

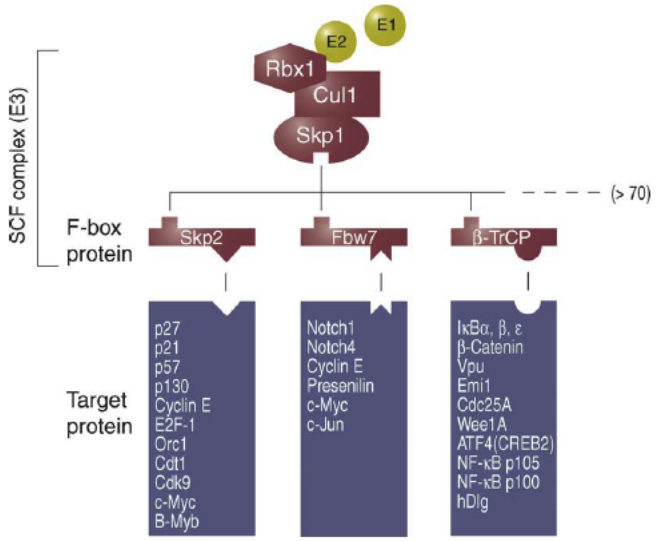
Charles H. Spruck* and Heimo M. Strohmaier *Cell Cycle 1:4, 250-254, July/August 2002* 65

The F-box consensus sequence



The F-box consensus sequence. The consensus was derived from the alignment of 234 sequences used to create the Pfam F-box profile [30]; the single-letter amino-acid code is used. Bold and underlined capital letters signify residues found in over 40% of the F-box sequences; bold, non-underlined, capital letters signify residues found in 20-40% of the F-boxes; bold lower case letters indicate residues found in 15-19% of the F-boxes; and non-bold lower case letters indicate residues found in 10-14% of the F-boxes. A minority of F-boxes contain small insertions in the alignment after positions 11 or 24, or small (1-3 residue gaps) at various locations. 66

Different F-box proteins target different set of substrates

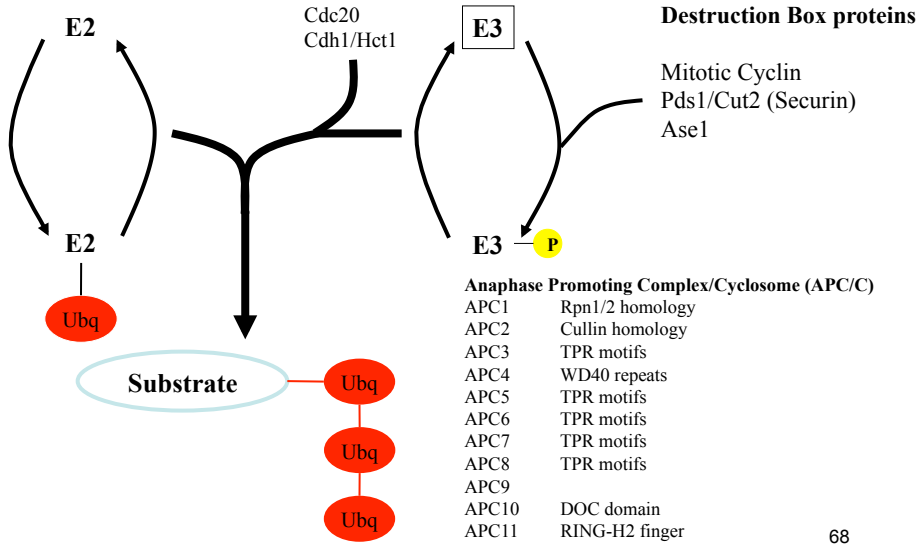


67

K.I. Nakayama, K. Nakayama / Seminars in Cell & Developmental Biology 16 (2005) 323–333

Ubquitination : Mitosis

UBC (UBC10/UBC4)



Destruction Box proteins

Mitotic Cyclin
Pds1/Cut2 (Securin)
Ase1

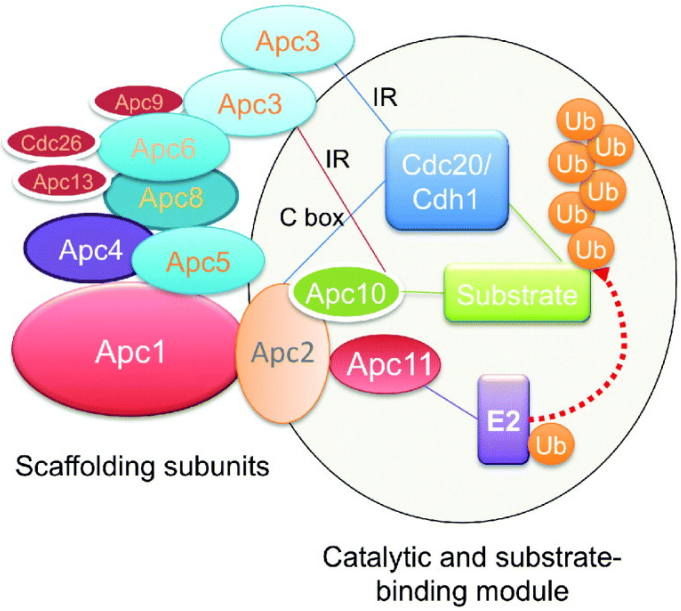
Anaphase Promoting Complex/Cyclosome (APC/C)

APC1	Rpn1/2 homology
APC2	Cullin homology
APC3	TPR motifs
APC4	WD40 repeats
APC5	TPR motifs
APC6	TPR motifs
APC7	TPR motifs
APC8	TPR motifs
APC9	
APC10	DOC domain
APC11	RING-H2 finger

68

Peters, JM., 2002, Mol Cell, 8, 931-942; Harper et al., 2002, Genes Dev., 16, 2179-206.

Structure of the APC/C ligase complex



Barford, Quaterly Reviews of Biophysics, 2011

69

What confers substrate specificity on the APC/C?

Cdc20
and Cdh1?

Both binds to TPR domain proteins

70

Cdc20 and Cdh1 modulate the substrate specificity of the APC/C

Cdc20

Only recognizes Destruction box

Only binds phosphorylated APC/C

Regulated by
- the mitotic checkpoint
- Emi1(Early mitotic inhibitor)
- Rca1(Regulator of Cyclin A1)
- Proteolysis *by Cdh1*

Cdh1

Recognizes D-box and KEN box

Binds unphosphorylated APC/C

Regulated by
- phosphorylation (by CDKs)
- Rca1

Substrates (KEN box)

RMSKY**KEN**KSENKKTVPQ	*H. sapiens* Sgo1
SGVSTN**KEN**EGP**P**EYPTKIE	*S. cerevisiae* Hsl1
MVNTDN**KEN**EP**P**NMEKAHM	*S. pombe* Mes1
NNLLDD**KEN**QD**P**SSQQFGA	*S. cerevisiae* Clb2
MPANED**KEN**NIVYTGNESS	*S. cerevisiae* Pds1
NNPSQV**KEN**LS**P**AKICPYE	*S. cerevisiae* Acm1
ASFLLS**KENQPEN**SQTPTK	*H. sapiens* Cdc20

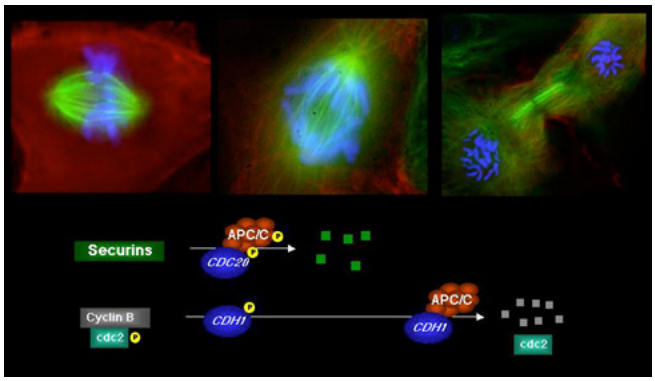
71

Table 1 | **Cell-cycle targets of ubiquitin-mediated proteolysis**

Substrate	Organism	Ligase	Specificity factor	Cell-cycle function	References
Securin/Pds1	H.s., S.c., others	APC/C	Cdc20	anaphase inhibitor	42,105
Clb2	S.c.	APC/C	Cdc20, Cdh1	cyclin B (mitosis)	41,42
Clb5	S.c.	APC/C	Cdc20	cyclin B (S phase)	106
Cyclin B	metazoan	APC/C	Cdc20, Cdh1	mitosis	61,107–111
Cyclin A	metazoan	APC/C	Cdc20, Cdh1	S phase, mitosis	107–109
Cdc20	H.s., S.c.	APC/C	Cdh1	mitosis	66–68
Plk/Cdc5	H.s., S.c.	APC/C	Cdh1	mitosis	64,68,113
Aurora A	H.s.	APC/C	Cdh1	mitosis	114,115
Dbf4	S.c.	APC/C	Cdc20	S phase	116
Ase1	S.c.	APC/C	Cdh1	mitotic-spindle dynamics	42,117
Nek2A	H.s.	APC/C	Cdh1	centrosome development	66
Cdc6	H.s.	APC/C	Cdh1	replication	118
Geminin	metazoan	APC/C	Cdh1	replication licensing	119,120
Cin8, Kip1	S.c.	APC/C	Cdh1	mitotic-spindle motor	121,122
Xkid	X.l.	APC/C	Cdh1	mitotic-spindle motor	123
Hsl1	S.c.	APC/C	Cdc20, Cdh1	G2–M transition	71,124
Sic1/Rum1	S.c., S.p.	SCF	Cdc4, Pop1/2	G1–S-transition Cdk inhibitor	81,82,125,126
Far1	S.c	SCF	Cdc4	G1–S-transition Cdk inhibitor	97
Cdc6/Cdc18	S.c., S.p.	SCF	Cdc4, Pop1/2	DNA replication	100,125,126
Swe1	S.c.	SCF	Met30	mitosis inhibitor	86
Cln1,2	S.c.	CF	Grr1	G1 cyclins	74,84,85
Glc1,2	S.c.	SCF	Grr1	budding	127
Cyclin E	H.s., D.m.	SCF	Cdc4, Ago	G1–S cyclin	54–56
$p27^{Kip1}$	H.s., M.m.	SCF	Skp2	G1–S-transition Cdk inhibitor	16–18
$p21^{Cip1}$	H.s.	SCF	Skp2	G1–S-transition Cdk inhibitor	28
p130	H.s., M.m.	SCF	Skp2	G1–S-transition inhibitor	27,128
Orc1	H.s.	SCF	Skp2	DNA replication	129
Emi1	M.m.	SCF	β-TrCP	mitosis (APC/C) inhibitor	88,89
Wee1	X.l.	SCF	Tome-1	mitosis inhibitor	129

D.m., *Drosophila melanogaster*; H.s., *Homo sapiens*; M.m., *Mus musculus*; S.c., *Saccharomyces cerevisiae*; S.p., *Schizosaccharomyces pombe*; β-TrCP, β-transducin-repeat-containing protein; X.l., *Xenopus laevis*.

72

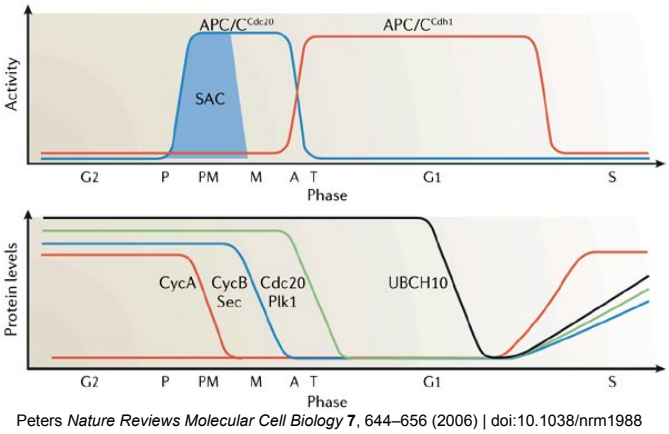


http://king.med.harvard.edu/node/79

Cdc20 activates the APC/C toward its substrates cyclin B and securin.

- Degradation of the cyclin B is required for eventual exit from mitosis.
- Degradation of the securin promotes sister chromatid separation and the metaphase to anaphase transition.

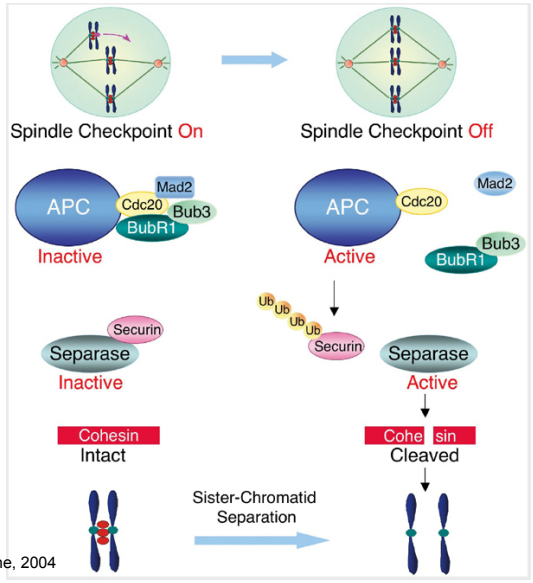
73



Peters *Nature Reviews Molecular Cell Biology* **7**, 644–656 (2006) | doi:10.1038/nrm1988

Anaphase promoting complex/cyclosome ^{Cdc20} (APC/C^{Cdc20}) is thought to be assembled in prophase (P) and initiates the degradation of cyclin A (CycA) already in prometaphase (PM). Proteolysis of cyclin B (CycB) and the separase inhibitor securin (Sec) also depends on APC/C^{Cdc20} but is delayed until metaphase (M) by the spindle-assembly checkpoint (SAC). During anaphase (A) and telophase (T), APC^{Cdh1} is activated, contributes to the degradation of securin and cyclin B, and mediates the destruction of additional substrates such as Polo-like kinase-1 (Plk1) and Cdc20, which leads to the inactivation of APC/C^{Cdc20}. In G1 phase, APC/C^{Cdh1} mediates the destruction of the ubiquitin-conjugating (E2) enzyme UBCH10, and thereby allows for the accumulation of cyclin A, which contributes to the inactivation of APC/C^{Cdh1} at the transition from G1 to S phase.

74



Bharadwaj and Yu, Oncogene, 2004

APC/C-Cdc20 mediates sister chromatid separation. APC/C-Cdc20 ubiquitinates securin and thereby marks it for proteolysis by the proteasome. This liberates the separase from its inhibitor and allows it to mediate the proteolytic cleavage of cohesin. Cleavage and disappearance from chromosomes of cohesin results in the loss of cohesion between sister chromatids. Lagging chromosomes and spindle damage block the activity of APC/C-Cdc20, which requires association of MCC complex with Cdc20.

75

Summary IV:

Key concept – cell cycle is regulated by Cdk/cyclin kinase activities as well as periodic synthesis and irreversible proteolysis of key regulatory proteins

1. Cell division is propelled by the oscillation of cyclin-dependent kinase (Cdk) activities, which in turn are regulated by the periodic synthesis and degradation of their regulatory subunits, cyclins.
2. The proteolytic destruction of cyclins as well as other cell cycle regulators (p27, p53, cdc20, securin etc.) ensure the unidirection progression of the cell cycle.
3. The SCF complex is a E3 ubiquitin ligase consisting of Skp1, Cullin, a F-box protein and Roc1. F-box proteins bind to specific substrates and recruit them to the SCF core through interaction of their F-box motif with Skp1. Cul1 mediates the interaction between the substrate and the E2. Roc1 is a RING finger protein that is the essential catalytic subunit of the ubiquitin ligase.

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Summary IV (cont.):

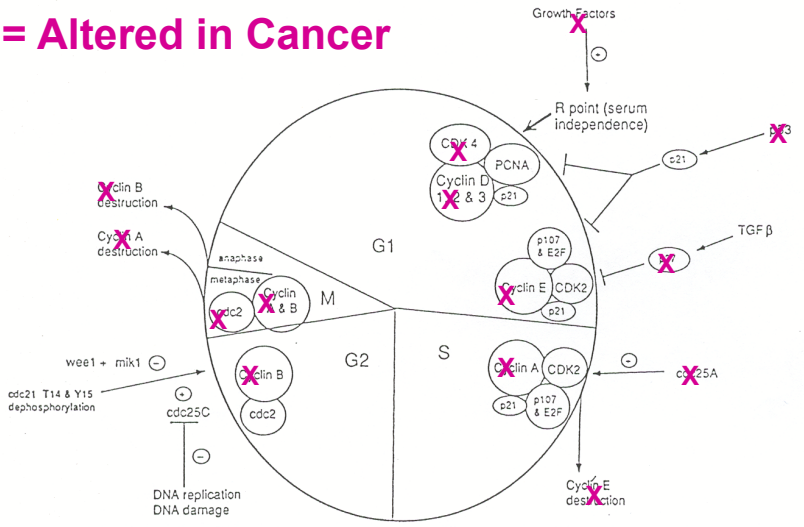
4. SCF is responsible for the degradation of G1 cyclins and p27.

Exit from mitosis and the M-phase switch.

- In the G2-phase, the cell is in a state of high cyclin/Cdk kinase activity and low APC activity.
- Accumulation of the APC activator, Cdc20, beginning in late S- and G2- phase triggers a reversal of this state.
- Cdc20 activates the APC toward its substrates cyclin B and securin.
  - ➢Degradation of the cyclin B is required for eventual exit from mitosis.
  - ➢Degradation of the securin promotes sister chromatid separation and the metaphase to anaphase transition.

77

X = Altered in Cancer



Cell 7:573, 1994

78

Levels of Regulation

Each cyclin protein is synthesized at a discrete stage.

Ectopic expression!

79

Ectopic (unscheduled) Cyclin Expression

Cyclin B - expressed in G1 at high levels
Cyclin E - expressed in late S and G2/M

Observed in leukemia, lymphoma, colon and breast cancers.

As a result:

Ectopically expressed cyclins are free to interact with their CDK partners throughout the cell cycle.

Substrates can by phosphorylated regardless of cell cycle position.

BYPASS OF CONTROL MECHANISMS

80

Levels of Regulation

Cyclin levels tightly regulated.

Cyclin over-expression!

81

Cyclin Over-Expression

Cyclin D1

Over-expressed or amplified in a variety of human cancers.

Breast	50%
Head & Neck	43%*
Esophageal	30%
Bladder	15%
Liver	10%
S.C. Lung	10%

*over-expression at time of surgery associated with increased risk of recurrence

82

One Mechanism of Cyclin D1 Over-Expression

PRAD1(Cyclin D1)/*bcl1* translocation

common translocation in lymphomas [t(11;14)(q13;q32)]

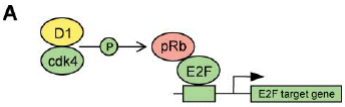
bcl1 is translocated adjacent to IgG heavy chain locus which is efficiently expressed in B-cell lymphomas

83

Consequences of overexpression of Cyclin D

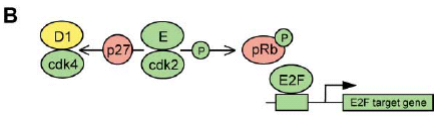
A) Direct catalytic effects:

- Activated CdK4/cyclin-D phosphorylates Rb proteins
- Release of E2F from Rb regulation
- Transcription of E2F downstream genes
- Cell cycle progression to S phase



B) Indirect non-catalytic effects:

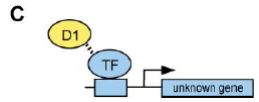
- Cdk4/cyclin-D complexes sequester p27
- Cdk2/cyclin-E complexes are free to phosphorylate Rb
- Activation of E2F regulated genes



84

Non-cell cycle effects:

C



C) Cyclin D1 modulates the activity of various transcription factors (TF) without the participation of cdks.

- Antagonizes C/EBPβ (CCAAT enhancer binding protein) binding to cyclin D (non-E2F) target genes
- Activates C/EBPβ-repressed genes

C C C T G G A A T	HSP70-2 (M59830)
T T C T G G A A A	KIAA0201 (D86956)
C T C T G G A A G	DnaJ homolog 1 (D85429)
T T C T G G A A G	HSC70 (Y00371)
C T C C A G A A G	DnaJ homolog 2 (L08069)
T T C C A G A A G	IEF SSP 3521 (M86752)
T T N N G A A A G G C T	**core of the consensus C/EBP recognition site**

Lamb et al., 2003. Cell 114:323-334.

85

Cyclin **A**

Over-expressed or amplified in a variety of human cancers.

Soft Tissue Sarcoma
Non-Hodgkin's Lymphoma
Astrocytoma
Hepatoma

Over-expression may result from failure to undergo ubiquitin-mediated degradation.

Over-expression leads to anchorage independence.

86

Prognostic Value of Cyclin A Over-Expression

Over-expression of cyclin A usually associated with a high rate of tumor cell proliferation.

Non-Hodgkin's Lymphoma :
low cyclin A ⟶ better prognosis

SOFT TISSUE SARCOMAS

high cyclin A ⟶ poor overall survival
⟶ poor metastasis-free survival

but

high cyclin A ⟶ better chemo. response
⟶ longer progression free survival

87

Cyclin **E**

Over-expressed in a variety of human cancers.

Breast	Kidney
Colon	Pancreas
Prostate	Some ALLs

Over-expression may result from failure to undergo ubiquitin-mediated degradation.

Over-expression in:
breast cancer = aggressive disease
testicular cancer = higher clinical stage, predictive of pulmonary metastases

88

Levels of Regulation

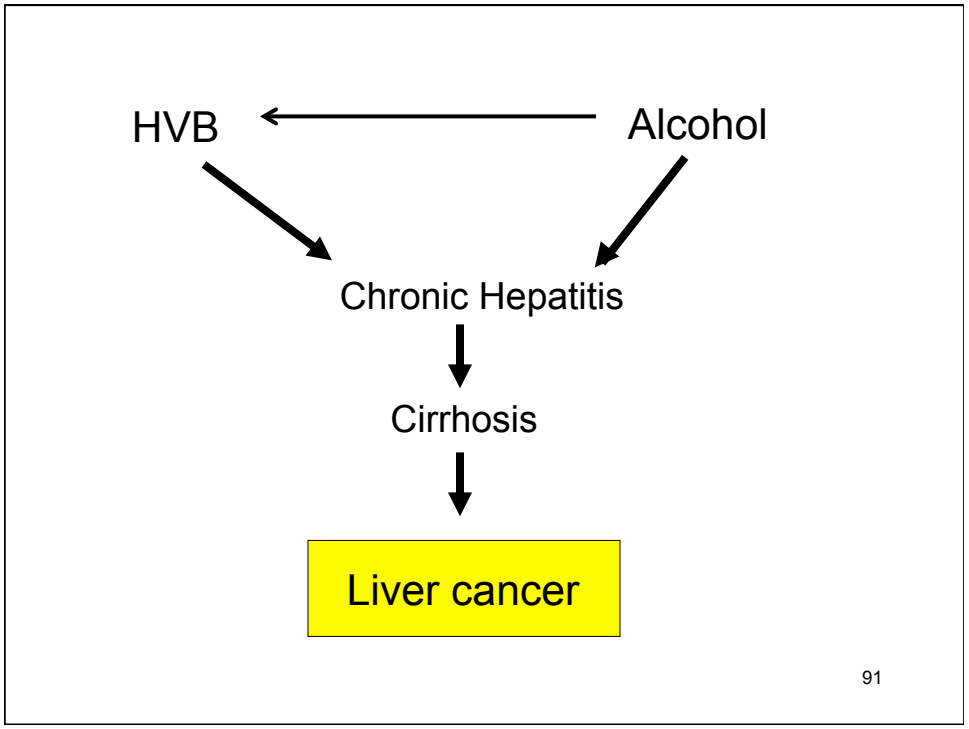
Cyclin degradation is regulated.

Cyclins not destroyed!

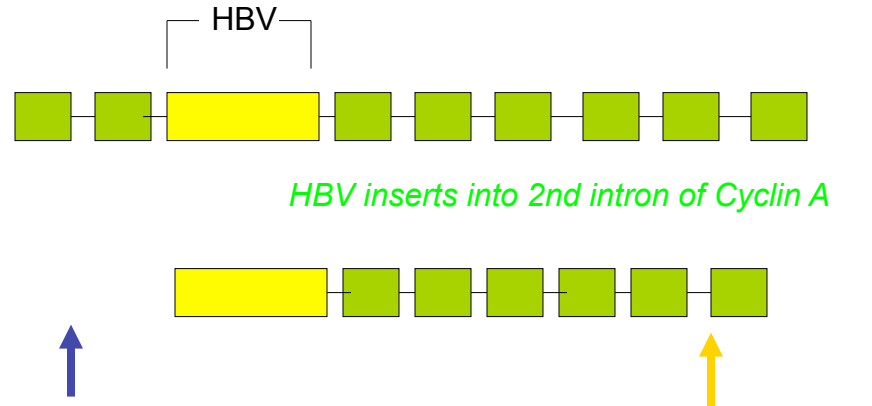
89

Over-expression of Cyclin A or Cyclin E may result from failure to undergo ubiquitin-mediated degradation.

90



Insertional Mutagenesis of Cyclin A in Liver Cancer



Curr. Opin. Genet. Dev. 3:11, 1993

92

Consequence:

Protein is *not* degraded

Cyclin A over-expressed

93

Levels of Regulation

Cyclin/CDK complexes are activated by regulated kinase activity.

Increased CAK Activity!

94

Increased CAK (Cdk7/cyclin H) Activity

Cdk7 *moderately* elevated in a variety of human tumor cell lines and biopsy specimens.

Retinoblastoma
Fibrosarcoma
Osteosarcoma
Cervix
Soft Tissue Sarcoma

Int. J. Cancer 66: 732, 1996

95

Levels of Regulation (cont.)

Deactivation of CDK activity by phosphorylation of ATP binding site or reactivation by phosphatases.

Phosphatase over-expression!

96

Phosphatase Over-Expression

cdc25A, cdc25B

mRNA and protein over-expressed in:

Aggressive Lymphomas (40-100%)
Head and Neck tumors

No over-expression in *indolent* lymphomas.

cdc25C levels relatively low in all cancers.

Cancer Res. 57:2366, 1997
Int. J. Cancer 89: 148, 2000

97

Levels of Regulation (cont.)

Activated kinases phosphorylate gene products required for transition to next phase.

De-regulation of G1 control!

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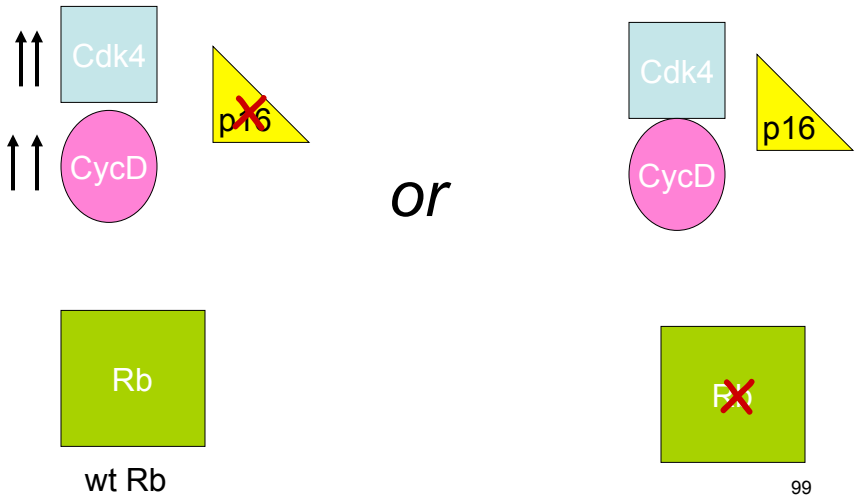
CDK4/Cyclin D, p16, Rb Pathway



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p16 Deletion (9p21)

Most common deletion in Glioblastoma (GBM)

More common in primary than secondary GBM

Associated with EGFR amplification

Not associated with p53 mutation

Confers significantly higher rates of proliferation, and poor prognosis

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A Cell Cycle Regulator Potentially Involved in Genesis of Many Tumor Types

Alexander Kamb,* Nelleke A. Gruis, Jane Weaver-Feldhaus, Qingyun Liu, Keith Harshman, Sean V. Tavtigian, Elisabeth Stockert, Rufus S. Day III, Bruce E. Johnson, Mark H. Skolnick

A putative tumor suppressor locus on the short arm of human chromosome 9 has been localized to a region of less than 40 kilobases by means of homozygous deletions in melanoma cell lines. This region contained a gene, Multiple Tumor Suppressor 1 (*MTS1*), that encodes a previously identified inhibitor (p16) of cyclin-dependent kinase 4. *MTS1* was homozygously deleted at high frequency in cell lines derived from tumors of lung, breast, brain, bone, skin, bladder, kidney, ovary, and lymphocyte. Melanoma cell lines that carried at least one copy of *MTS1* frequently carried nonsense, missense, or frameshift mutations in the gene. These findings suggest that *MTS1* mutations are involved in tumor formation in a wide range of tissues.

Science 264:436, 1994

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Table 1. Deletions in tumor cells and primary tumors.

Tumor type	Lines (*n*)	Deletions (*n*)	Deletions (%)
Astrocytoma	17	14	82
Bladder	15	5	33
Breast	10	6	60
Colon	20	0	0
Glioma	35	25	71
Leukemia	4	1	25
Lung	59	15	25
Melanoma	99	57	58
Neuroblastoma	10	0	0
Osteosarcoma	5	3	60
Ovary	7	2	29
Renal	9	5	56
Total	290	133	46

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Science 264:436, 1994

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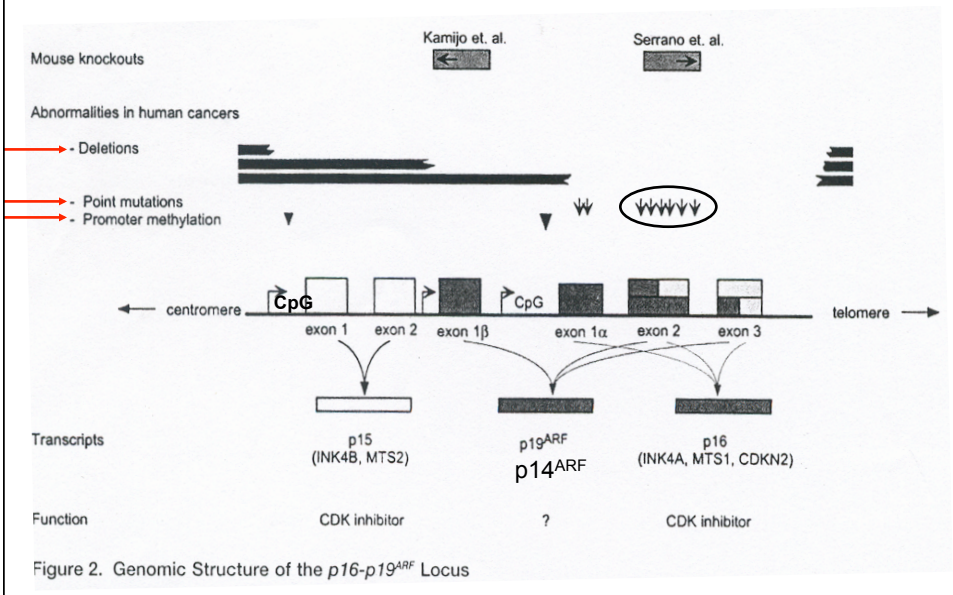

Figure 2. Genomic Structure of the *p16-p19*^{ARF} Locus

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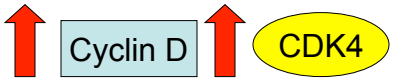

CDK4/Cyclin D Amplification

~10-15% high grade astrocytomas

No difference between primary and secondary GBM

Associated with mutated p53

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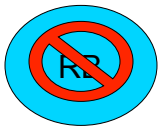

RB Inactivation (13q)

Second most common mutation in GBM

No Difference between primary and secondary GBM

Can be associated with p53 mutation/deletion

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Tumors With Inactivating *RB* Mutations

• Retinoblastoma	100%
• Osteosarcoma	90%
• Small cell lung carcinoma	90%
• Breast carcinoma	30%
• Bladder carcinoma	30%
• Malignant glioma	30%
• Leukemias	30%
• Cervical carcinoma	15%
• Pancreatic carcinoma	
• Prostate carcinoma	

Cobrinik, 2000

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Levels of Regulation (cont.)

CDK inhibitors block assembly of the complex or activation of kinase activity.

Inhibitors deleted!

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Inhibitors Deleted - Phenotypes of CKI-Deficient Mice

p21-/-	No development defect No increased cancer risk G1 checkpoint defect
p27-/-	Increased animal size and organ overgrowth (particularly spleen and thymus) Female infertility, Disorganization of retina Pituitary hyperplasia (adenoma) No defect in response to TGF-β

Trends Cell Biol. 6: 388, 1996

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Inhibitors Deleted in Cancer

p27 low or absent in a variety of human tumors

- Breast
- Colon
- Esophagus
- Gastric
- Lung
- Prostate
- Melanoma
- Thyroid
- Lymphoma

Am. J. Pathol. 154: 313, 1999.

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Inhibitors Deleted in Cancer

p27 In general, low or absent expression is correlated with:

- poor prognosis
  - (survival, treatment failure)
- biologically aggressive tumors

Am. J. Pathol. 154: 313, 1999.

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Inhibitors Deleted - Phenotypes of CKI-Deficient Mice

Ink4a-/-

Mild proliferative expansion of the spleen
High incidence of fibrosarcoma and lymphoma
Increased susceptibility to tumor induction by carcinogens
Increased sensitivity to transformation by Ha-Ras
Failure to senesce
Decreased doubling time

Trends Cell Biol. 6: 388, 1996

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Summary V : Dysregulation of the cell cycle

- Ectopic or unscheduled expression of cyclins
  - Cyclin B expressed in G1
  - Cyclin E expressed in late S and G2/M
  - Observed in leukemia, lymphoma, colon and breast cancers
  - By pass normal cell cycle control
- Overexpression of cyclins
  - Cyclin D1/*bcl1* translocation - [t(11;14)(q13;q32)] translocation commonly found in lymphomas
  - Cyclin D1 is overexpressed or amplified in a variety of human cancers
  - Overexpression of cyclin D1 causes:
    - Cdk4/cyclin-D phosphorylates Rb proteins
    - E2F activation and transcription of E2F target genes
    - Cell cycle progression to S phase
    - Cdk4/cyclin-D complexes sequester p27
    - Cdk2/cyclin-E phosphorylates Rb and E2F is activated
    - Cyclin D1 antigonizes C/EBPβ and activates C/EBPβ-repressed genes

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Summary cont'd: Dysregulation of the cell cycle

- Overexpression of cyclins cont'd
  - Overexpression of cyclin A:
    - Overexpressed and amplified in soft tissue sarcoma, non-hodgkin's lymphoma, astrocytoma, and hepatoma
    - Might result in failure to undergo ubiquitin-mediated degradation
    - Leads to anchorage independence
  - Overexpression of cyclin E
    - In breast, colon, prostate, kidney, pancreas and some ALLs
    - Might result in failure to undergo ubiquitin-mediated degradation
    - Correlates to aggressive disease in breast cancer and higher clinical stage and predictive of pulmonary metastases in testicular cancer

- Failure to degrade cyclin
  - Cyclin A and E might fail to undergo ubiquitin-mediated degradation
  - Insertional mutagenesis of cyclin A by Hepatitis B virus results in deletion of the destruction box - results in cyclin A overexpression.

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Summary cont'd: Dysregulation of the cell cycle

- increased CAK activity
  - Cdk7 elevated in a variety of cancers

- phosphatase overexpression
  - cdc25A and cdc25B are overexpressed in aggressive lymphomas, and head & neck tumors
  - Activated Cdks phosphorylate gene products required for transition to the next cell cycle phase

- deletion in the cdk4/cyclin-D, p16, Rb pathway
  - p16 deletion in 9p21
    - Common in 1° and 2° gliobastoma
    - Higher rates of proliferation and poorer prognosis
  - Cdk4/cyclin-D amplification in 10-15% of high grade astrocytomas
  - Rb inactivation in 13q
    - Second most common mutation in gliobastoma

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Summary cont'd: Dysregulation of the cell cycle

- Cdk inhibitors deleted
  - p27 expression is low or absent in a variety of cancers
  - low expression of p27 is correlated with poor prognosis and aggressive tumors
  - Ink4a inactivation is prevalent in pancreatic adenocarcinoma, transitional cell carcinoma of the bladder, melanoma, non-small cell lung cancer, glioma etc.

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Chemical inhibitors of Cdks

Inhibitor	IC_{50} (μM)	Reference no.
Purine analogues		
Dimethylaminopurine	120	Meijer & Pondaven 1988; Neant & Guerrier 1988
N6-isopentenyladenine	55	Rialet & Meijer 1991
Olomoucine	7	Vesely *et al.* 1994
Roscovitine	0.2–0.8	De Azevedo *et al.* 1997; Meijer *et al.* 1997
CVT-313	4.2	Brooks *et al.* 1997
Purvalanol A	0.004	Gray *et al.* 1998
Purvalanol B	0.006	Gray *et al.* 1998
New cytokinin analogues	0.1–3.8	Vermeulen *et al.* 2002a; Vermeulen *et al.* 2002b
Olomoucine II	0.02	Krystof *et al.* 2002
NU2058	5	Arris *et al.* 2000
Pyrimidine analogues		
NU6027	2.5	Arris *et al.* 2000
Butyrolactone	0.6	Kitagawa *et al.* 1993; Kitagewa *et al.* 1994
Flavonoïds		
Flavopiridol	0.4	Losiewicz *et al.* 1994
Oxoflavopiridol	0.130	Kim *et al.* 2000
Thioflavopiridol	0.110	Kim *et al.* 2000
Paullones		
Kenpaullone	0.4	Zaharevitz *et al.* 1999
Alsterpaullone	0.035	Schultz *et al.* 1999
Indolinones		
Indirubin	10˙	Hoessel *et al.* 1999
Indirubin-3′-monoxime	0.18	Hoessel *et al.* 1999
5-chloro-indirubin	0.4	Hoessel *et al.* 1999
Indirubin-5-sulphonic acid	0.055	Hoessel *et al.* 1999
SU9516	0.04	Lane *et al.* 2001
Staurosporine and derivatives		
Staurosporine	0.003–0.009	Gadbois *et al.* 1992
UCN-01	0.031–1	Wang *et al.* 1995; Kawakami *et al.* 1996
9-hydroxyellipticine	1	Ohasi *et al.* 1995
Suramin	4	Larsen 1993
Hymenialdisine	0.022	Meijer *et al.* 2000
Toyocamycin	0.88	Park *et al.* 1996

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