The roles of cyclin-dependent kinases (Cdks) in regulation of transcription and cell cycle

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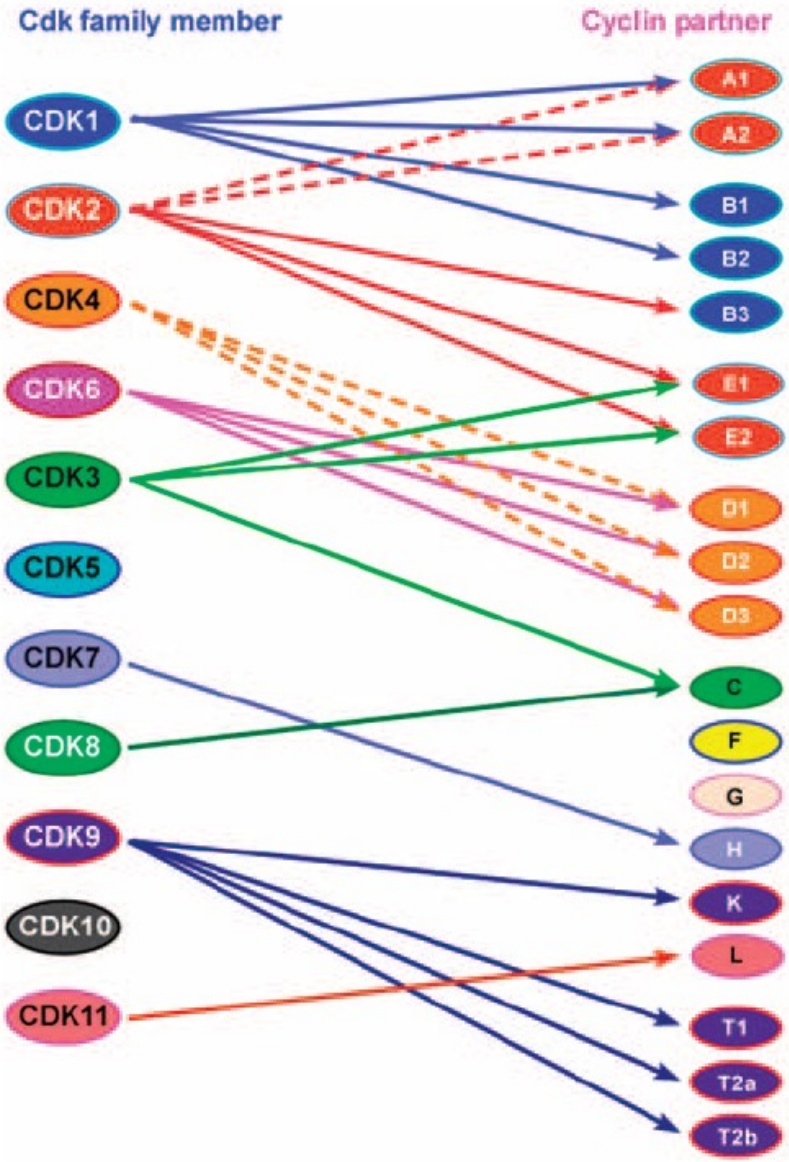
Cyclin-dependent kinases (Cdks)

Protein comlexes that compose of 1) Kinase subunit
2) Cyclin subunit

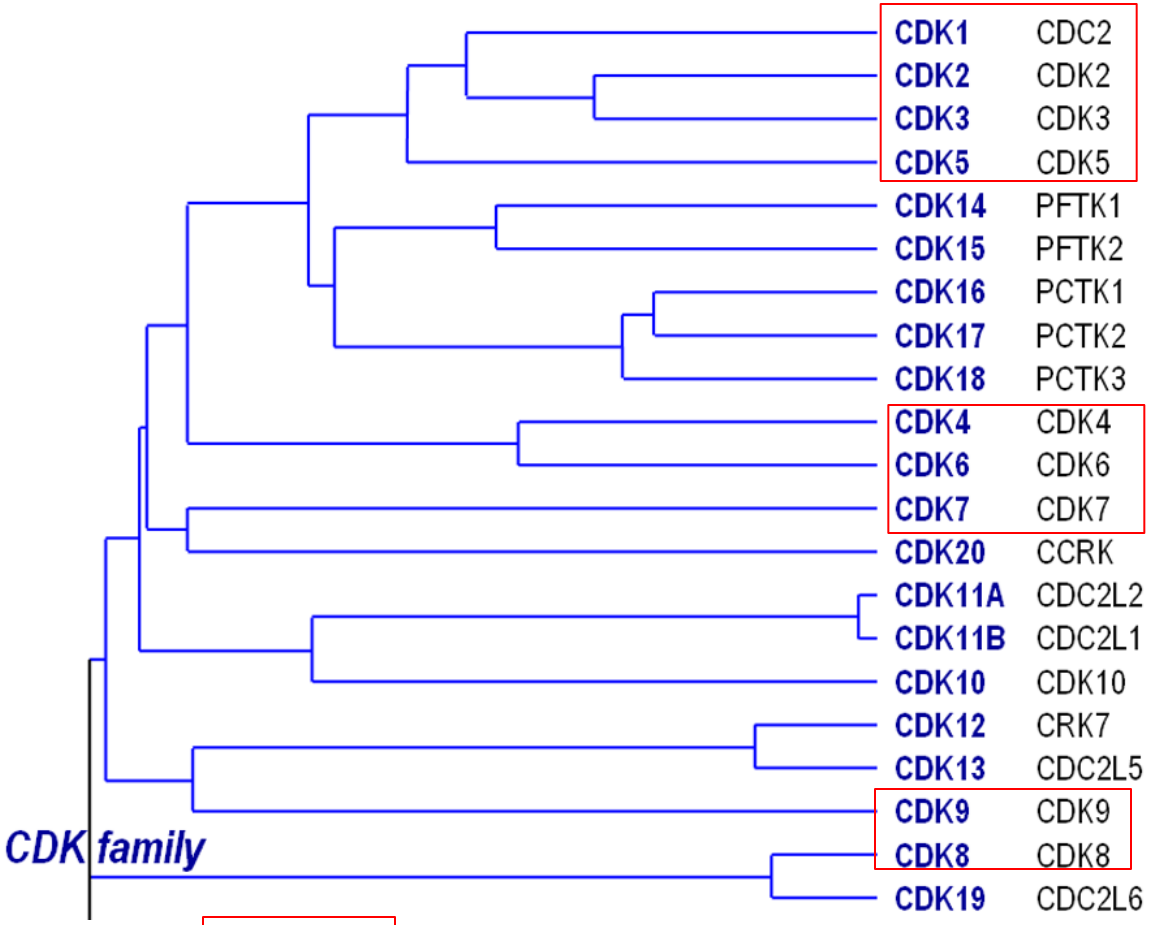
Serine-threonine kinases-regulate function of proteins by phosphorylation of either Serine (S) or Threonine (T)

Both subunits needed for the kinase activity of the complex

Most Cdks usually have at least one Cyclin partner



In humans there are at least 21 genes encoding Cdks however only about half of the Cdks are sufficiently studied



Human cell has 21 Cdks and 29 Cyclins

The Cdk complexes regulate various processes in cells

Major functions:

-Regulation of Cell Cycle (Cdk1,2,4,6,7)

-Regulation of Transcription (Cdk7,8,9,12)

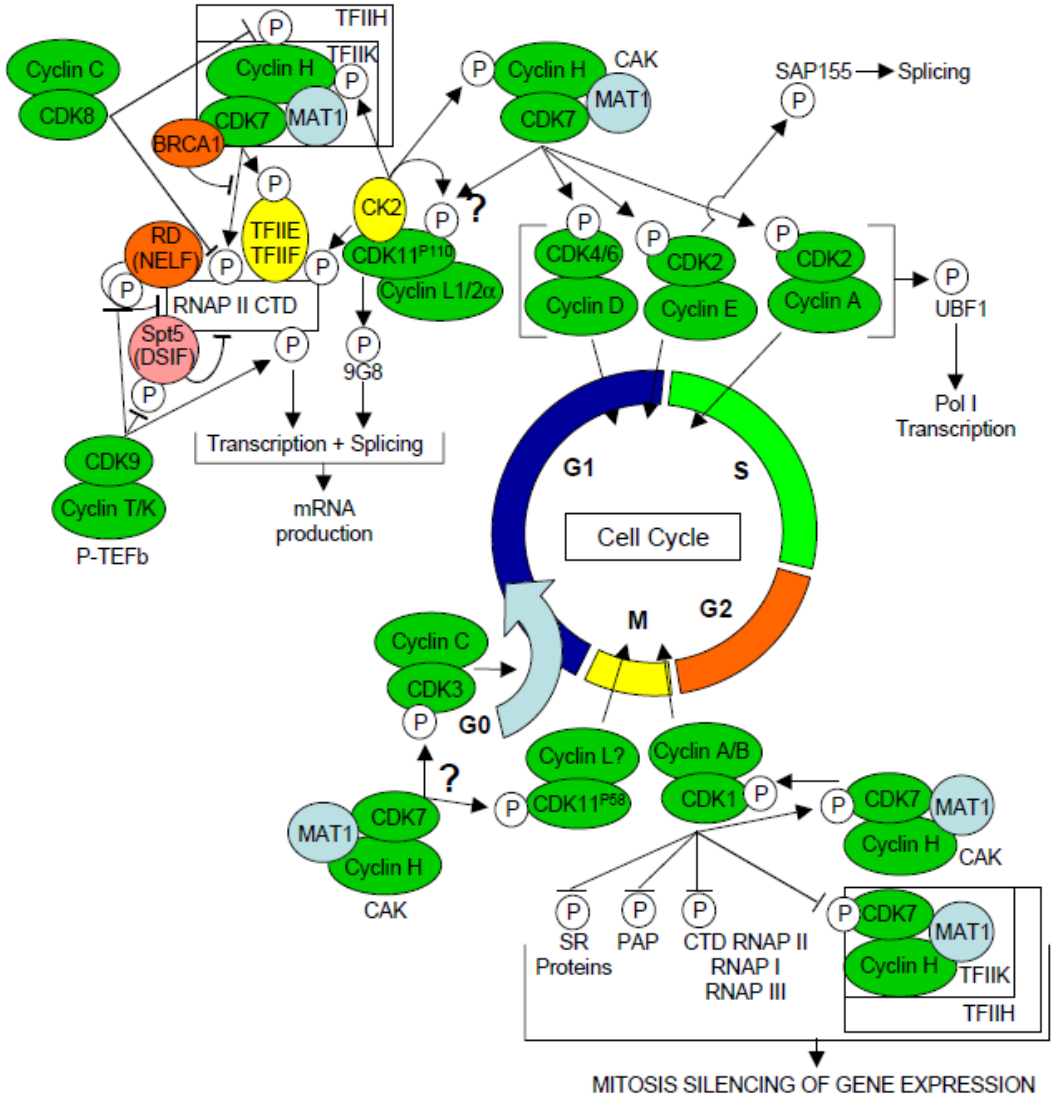
Other functions:

- regulation of pre-mRNA processing (Cdk11, Cdk9)

- regulation of neuronal cell differentiation (Cdk5)

- likely more functions to be discovered

Cdk complexes regulate various processes in cells



Regulation of kinase activity of Cdk complexes-overview

Activation of Cdk kinase activity:

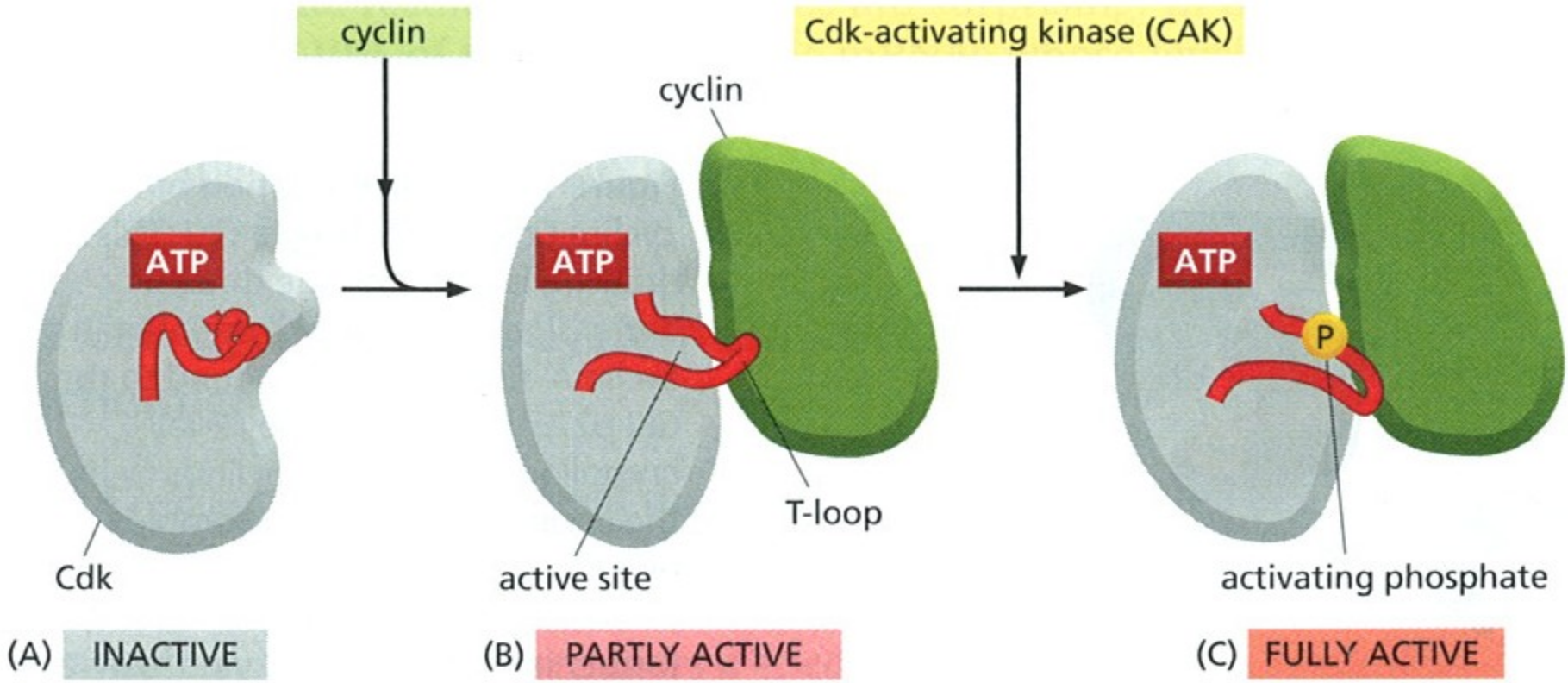
-Association of Cdk with various Cyclin subunits
-Phosphorylation of threonine in the “T-loop” of Cdk
-Degradation of Cdk inhibitor proteins by ubiqitination and proteolysis

Inhibition of Cdk kinase activity:

-Binding of Cdk inhibitor proteins to Cyc/Cdk complexes
-Inhibitory phosphorylation of Cdk
-Ubiqitination and degradation of Cyclins in proteasome
-Binding of Cdk inhibitor proteins together with small nuclear RNA to Cyc/Cdk complex

Activation of Cdk kinase activity:

-Association of Cdk with various Cyclin subunits
-Phosphorylation of Threonine in the "T-loop" of Cdk



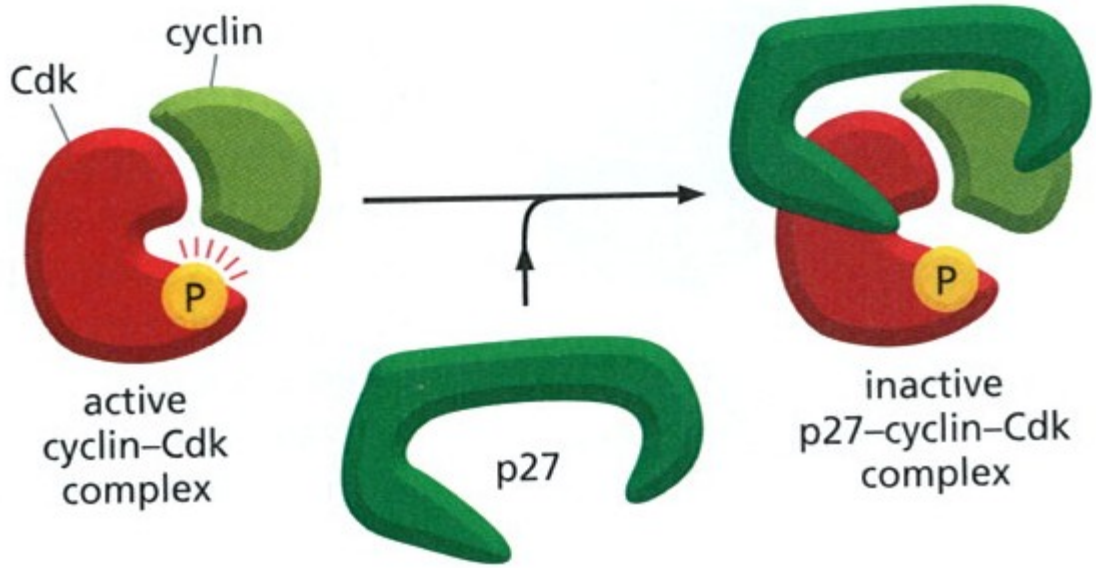
T-loop blocks active site (active site=ATP binding site)

T-loop moves out of the active site

P-T-loop improves binding of substrate

Inhibition of Cdk kinase activity:

-Binding of Cdk inhibitor proteins to Cyc/Cdk complexes

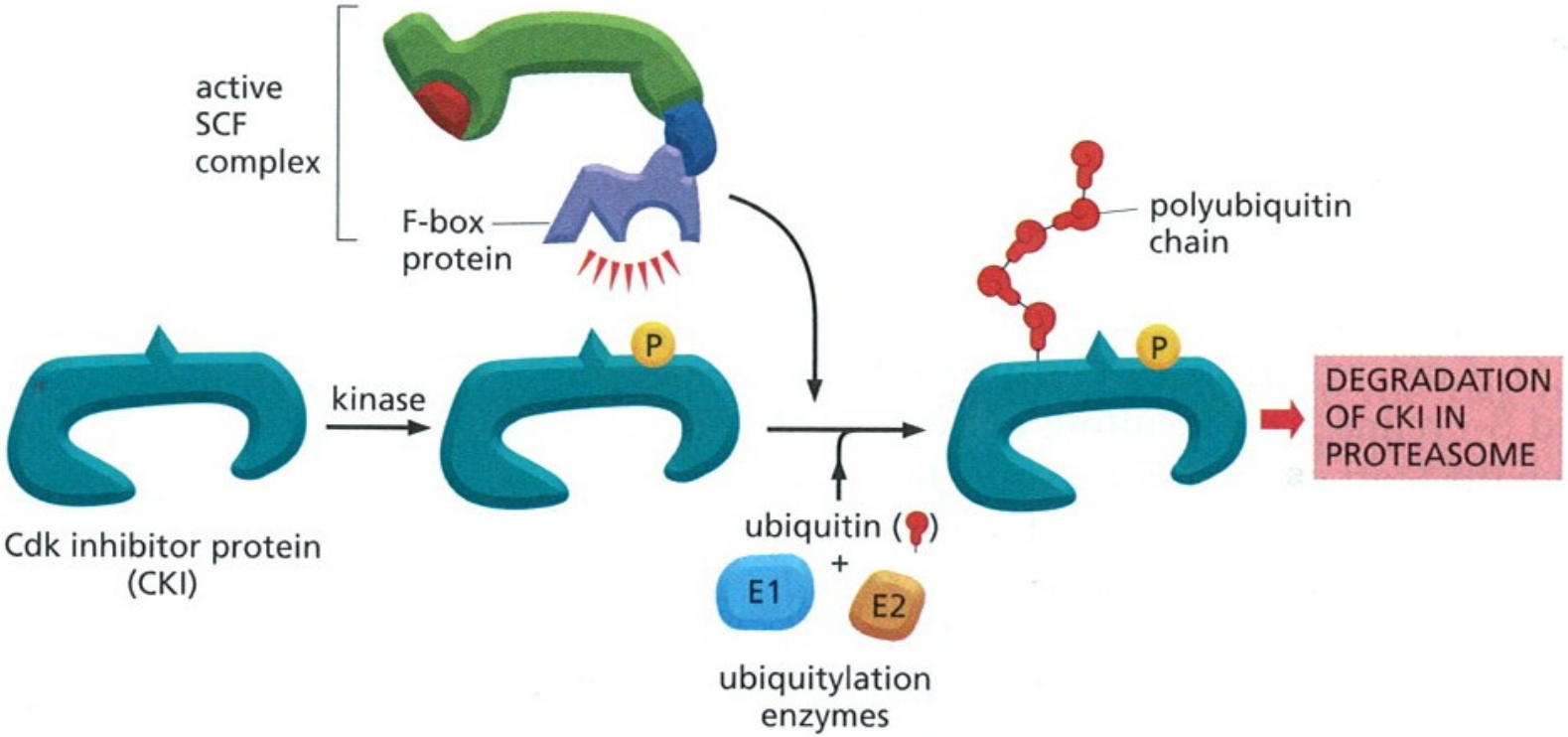


P27 binding distorts and binds into the active site of Cdk2 (for example inhibits G1/S-Cdk in G1 phase)

Cdk inhibitor proteins (CKIs)	
Sic1 (budding yeast)	suppresses Cdk1 activity in G_1; phosphorylation by Cdk1 at the end of G_1 triggers its destruction
p27 (mammals)	suppresses G_1/S-Cdk and S-Cdk activities in G_1; helps cells withdraw from cell cycle when they terminally differentiate; phosphorylation by Cdk2 triggers its ubiquitylation by SCF
p21 (mammals)	suppresses G_1/S-Cdk and S-Cdk activities following DNA damage
p16 (mammals)	suppresses G_1-Cdk activity in G_1; frequently inactivated in cancer

Activation of Cdk kinase activity:

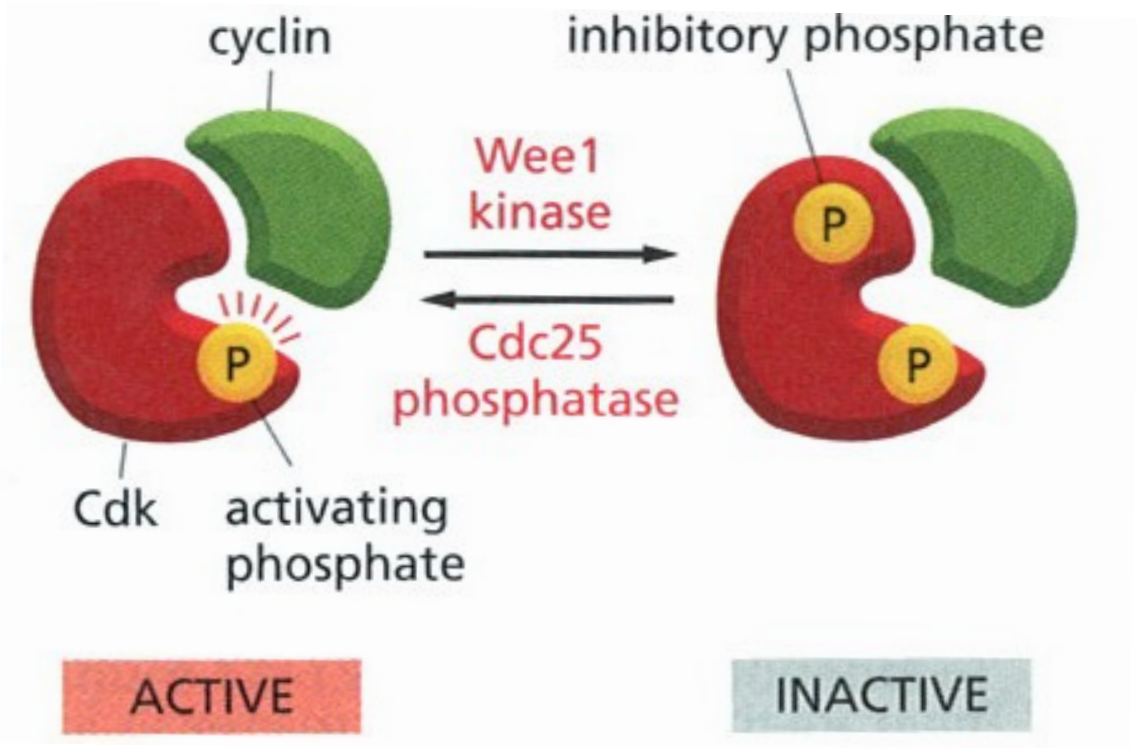
-Degradation of Cdk inhibitor proteins by ubiqitination and proteolysis



Cell cycle-dependent phosphorylation of Cdk inhibitor is a "mark" for recognition by SCF ubiquitin ligase, ubiquitinylation and degradation, rendering Cyc/Cdk complex more active

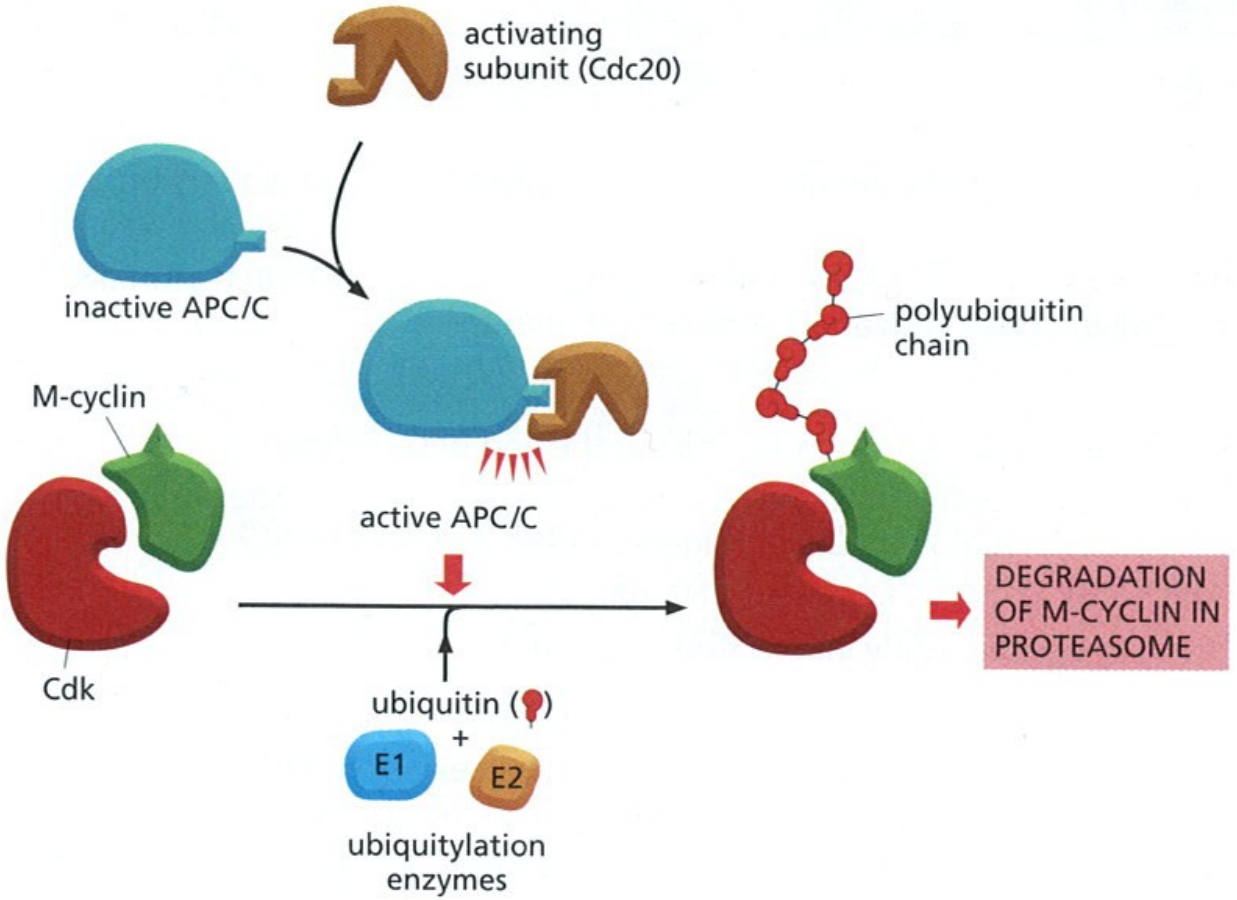
Inhibition of Cdk kinase activity:

-Inhibitory phosphorylation of Cdk



Inhibition of Cdk kinase activity:

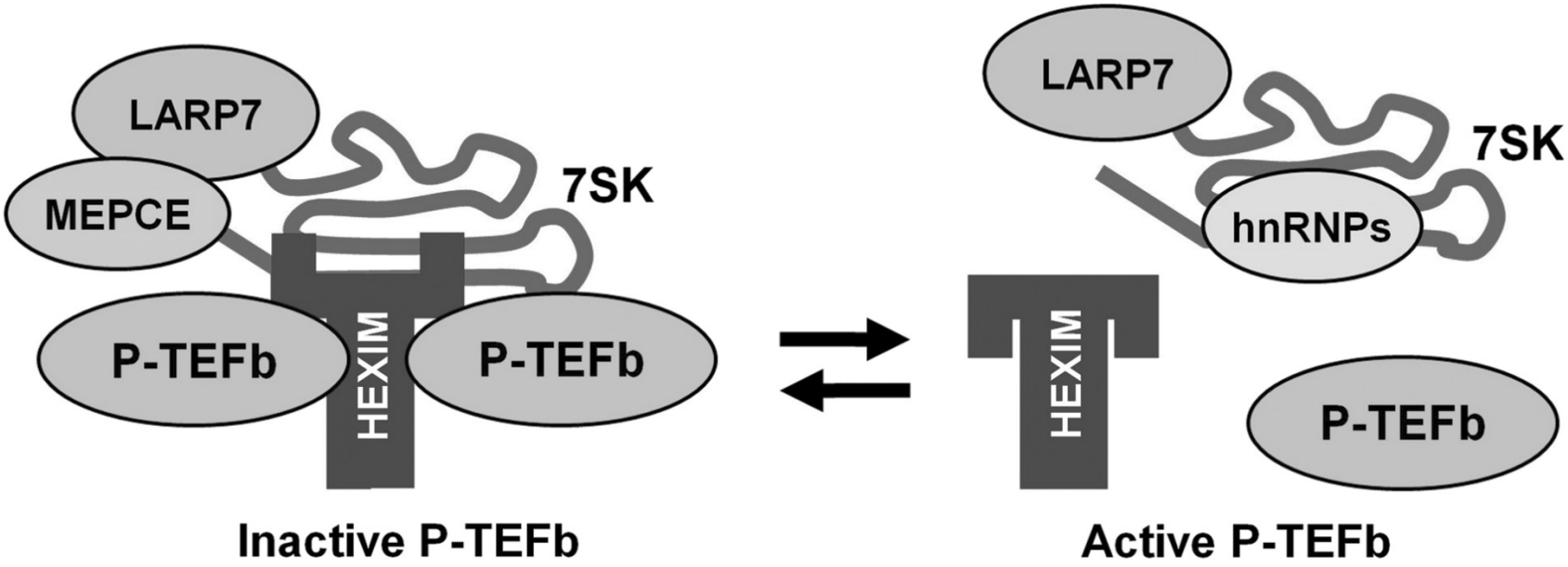
-Ubiquitination and degradation of Cyclin by proteasome



Mitosis-dependent activation of APC ubiquitin ligase leads to ubiquitination of Cyclin and its degradation

Inhibition of Cdk kinase activity:

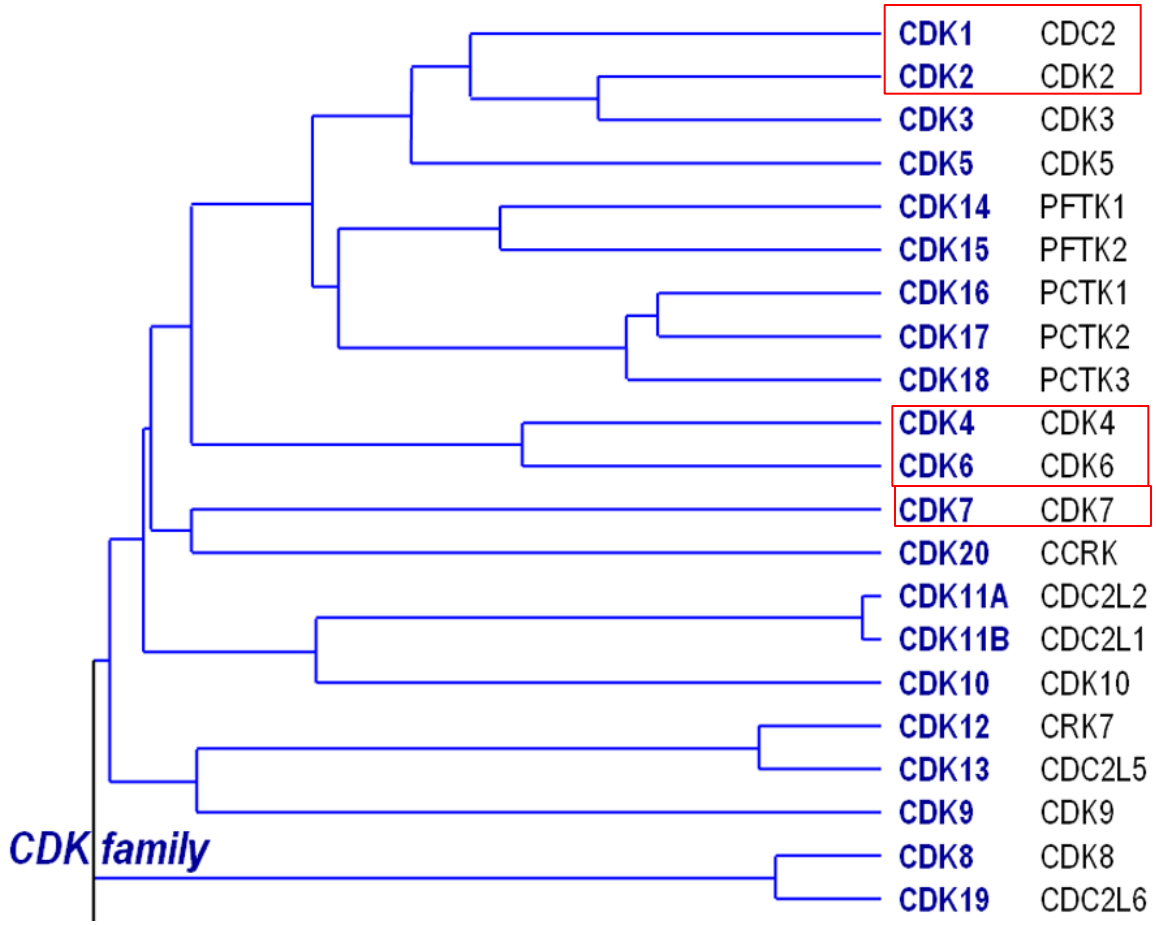
-Binding of Cdk inhibitor proteins and 7SK small nuclear RNA (7SK snRNA) to CycT/Cdk9 complex



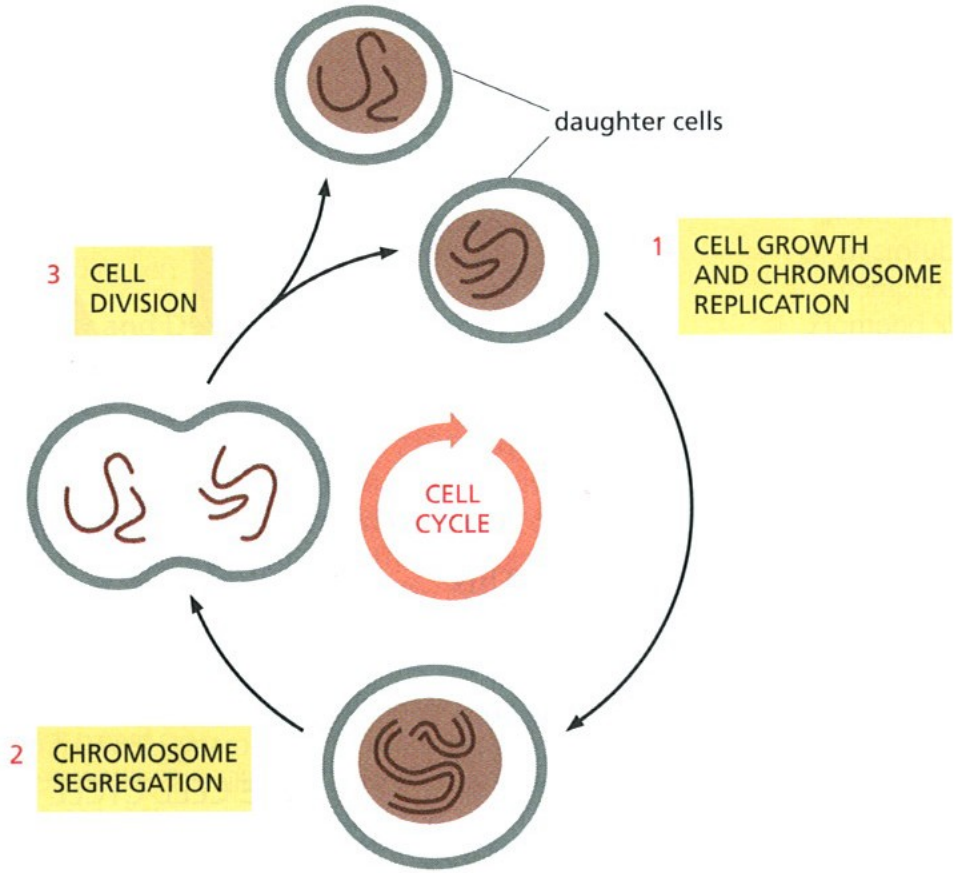
P-TEFb=Cdk9

The kinase activity of Cdk9 is inhibited by binding to several proteins and small nuclear RNA, 7SK snRNA

Regulation of Cell Cycle by Cdks

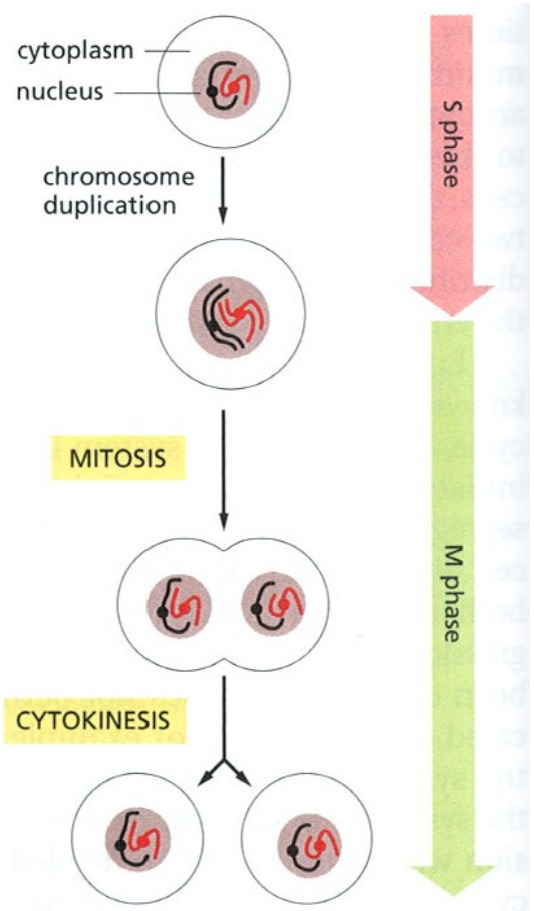


Cell Cycle



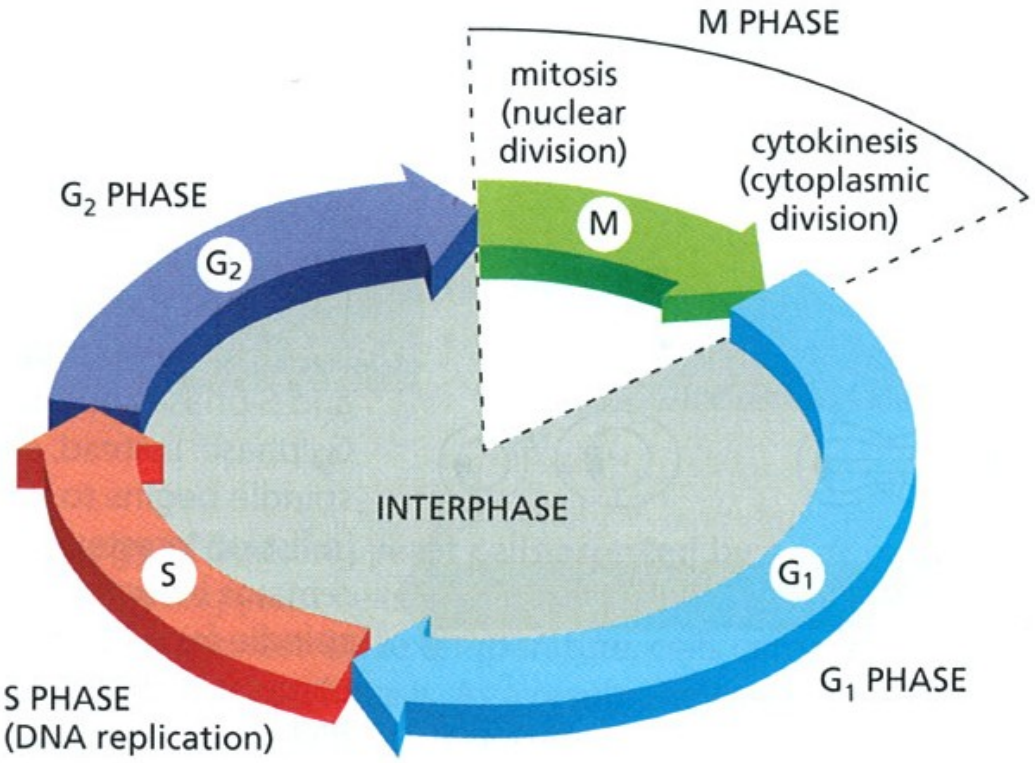
Cell cycle leads to production of two genetically identical daughter cells

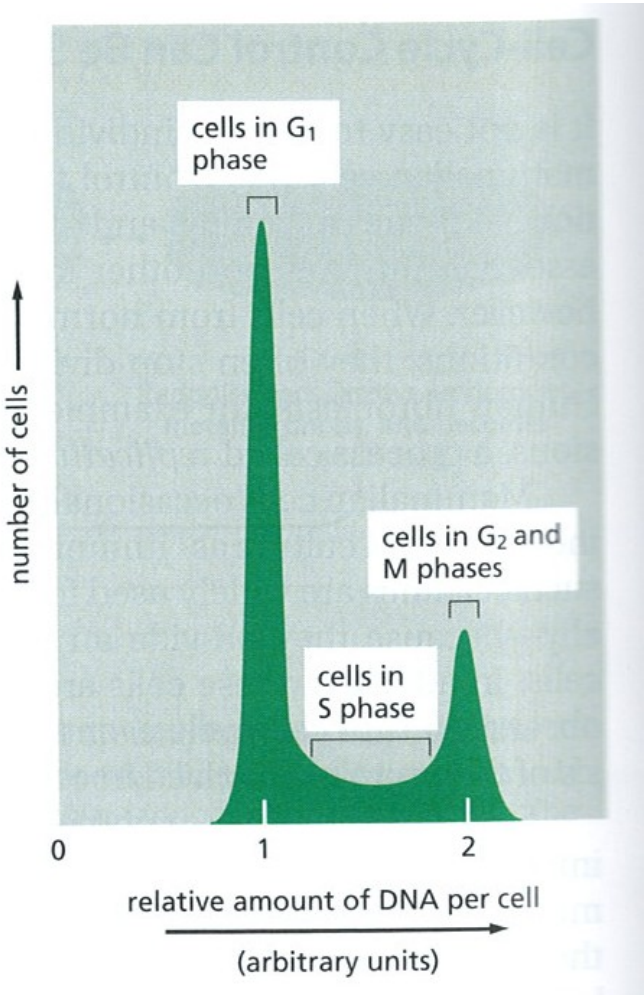
Major events of the cell cycle



S-phase – DNA synthesis-duplication of the chromosomes
M-phase – mitosis-pair of chromosomes segregated into the nuclei
– cytokinesis- the cell divides into two identical cells

The cell cycle has four phases

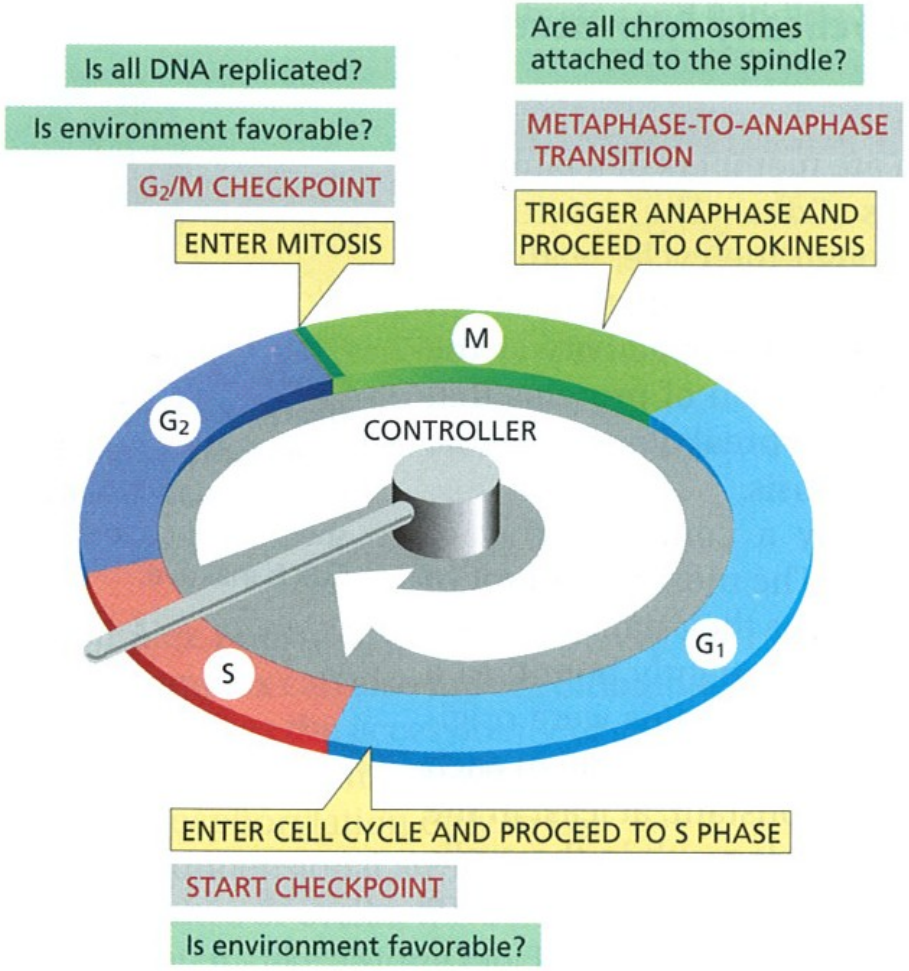




G1 and G2 phases-time delay to allow the growth of the cell

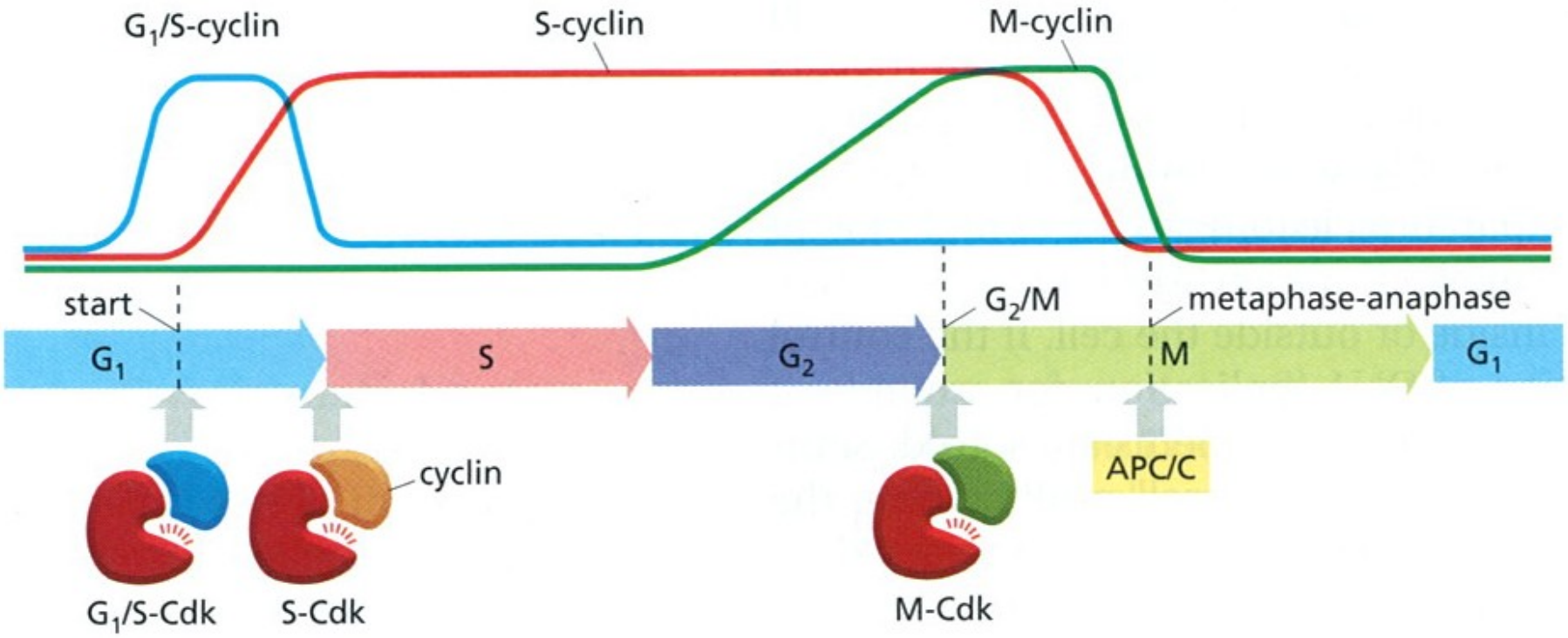
-time to monitor external and internal conditions before commitment to onset of S and M phase

The control of the cell cycle-three major checkpoints



Control of the cell cycle triggers essential processes such as DNA replication, mitosis and cytogenesis

Cell cycle control system depends on cyclically activated Cdks



Cyclin protein levels change, Cdk protein levels are constant

Cyclical changes (expression and degradation) in Cyclin protein levels result in cyclic assembly/disassembly and activation/inhibition of Cyc/Cdk complexes;
this leads to phosphorylation/dephosphorylation of proteins that initiate and regulate cell cycle events

Major Cyclins and Cdks in Vertebrates and Yeast

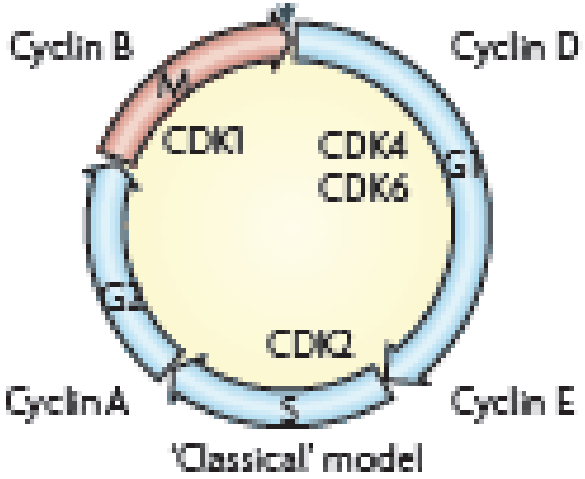
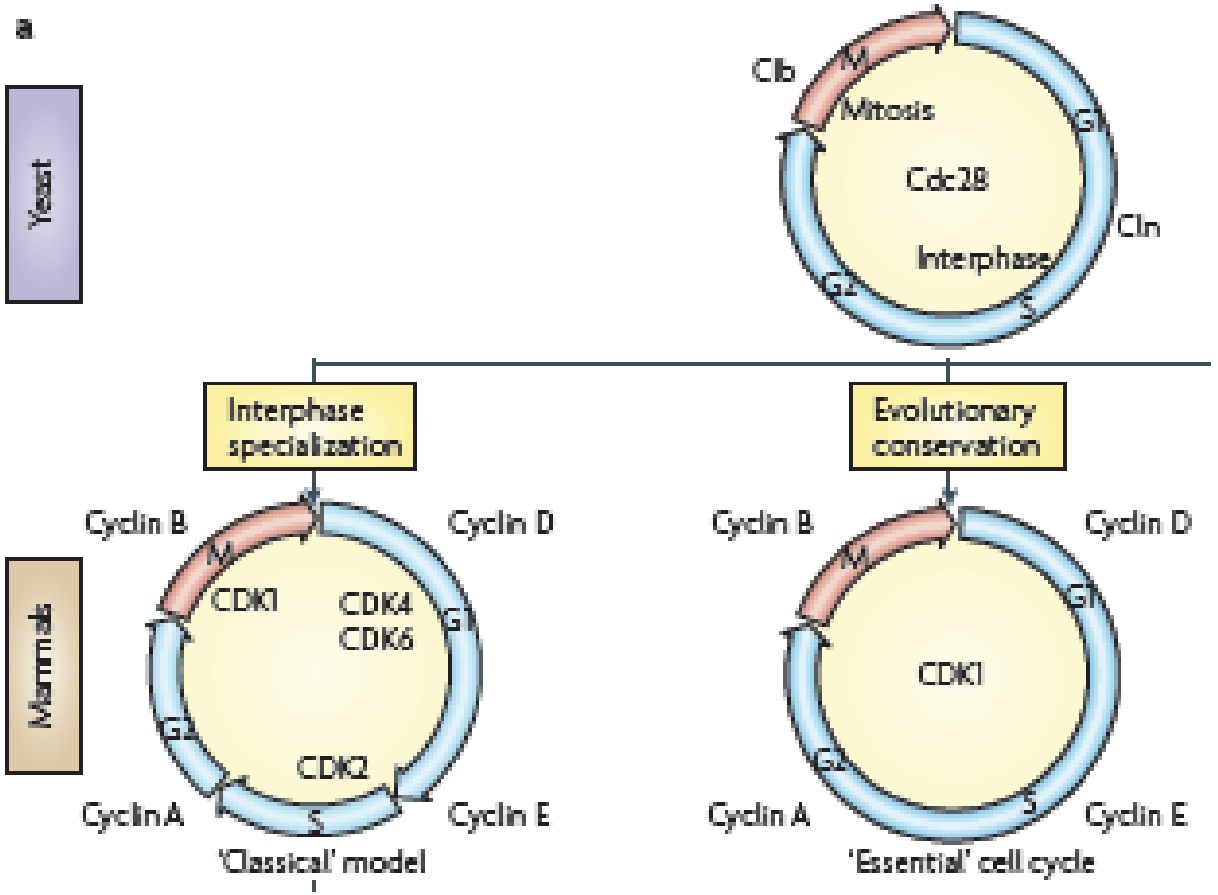


Table 17–1 The Major Cyclins and Cdks of Vertebrates and Budding Yeast

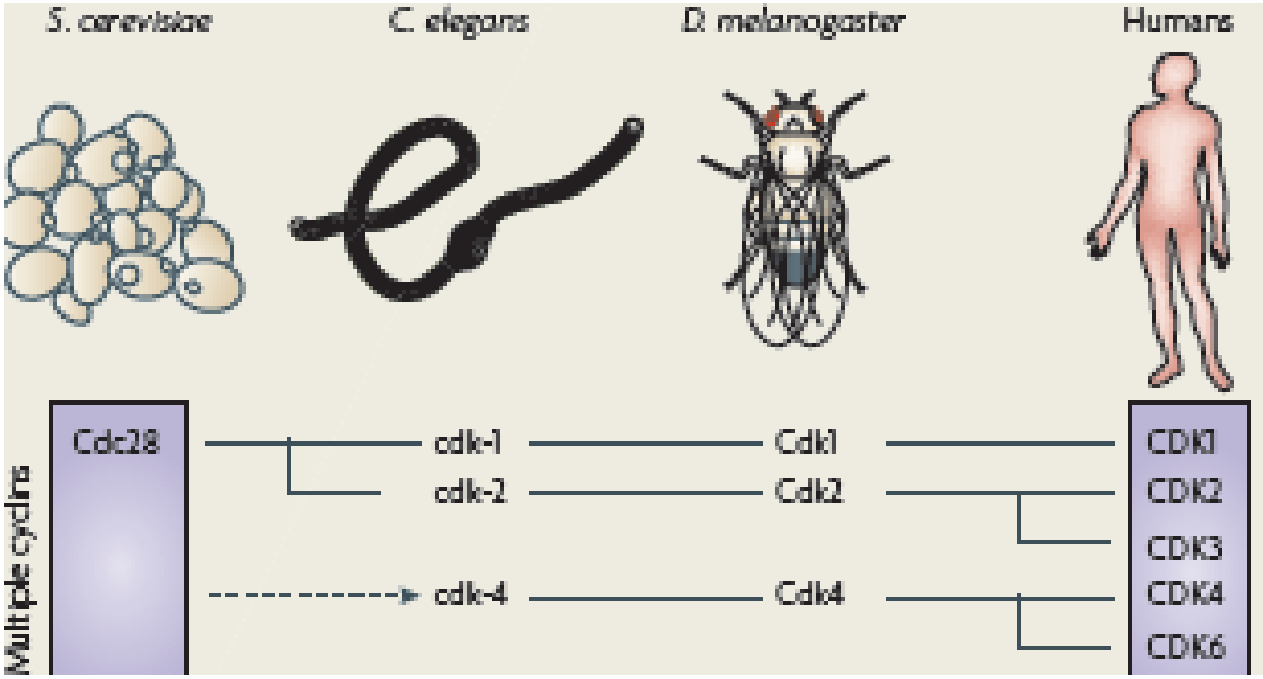
CYCLIN–CDK COMPLEX	VERTEBRATES		BUDDING YEAST	
	CYCLIN	CDK PARTNER	CYCLIN	CDK PARTNER
G_1-Cdk	cyclin D*	Cdk4, Cdk6	Cln3	Cdk1**
G_1/S-Cdk	cyclin E	Cdk2	Cln1, 2	Cdk1
S-Cdk	cyclin A	Cdk2, Cdk1**	Clb5, 6	Cdk1
M-Cdk	cyclin B	Cdk1	Clb1, 2, 3, 4	Cdk1

Comparison of the yeast and mammalian cell cycle

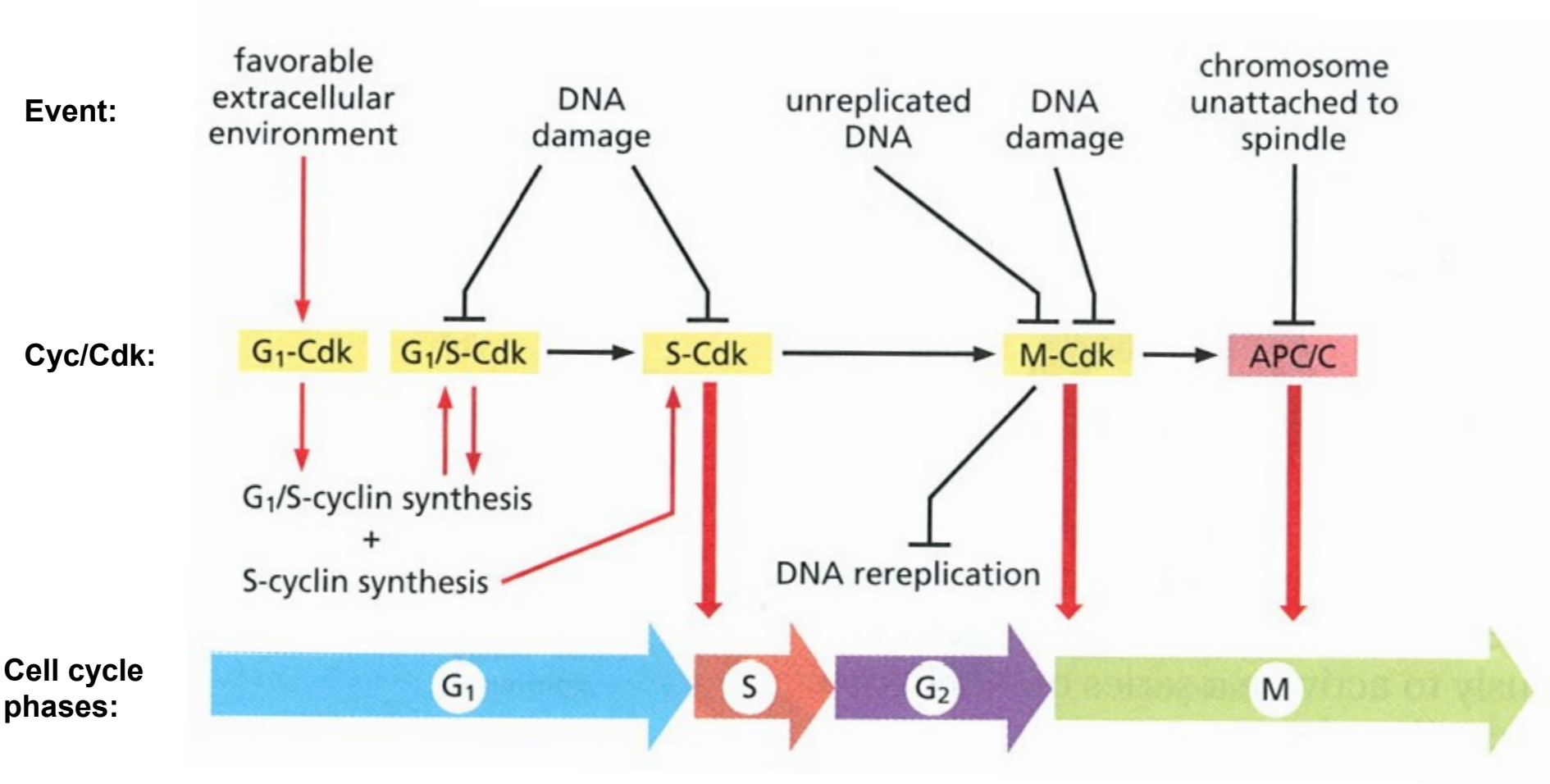


Yeast- cell cycle is directed by one Cdk-Cdk1 (cdc28)
Mammals-several Cdks (classical model), Cdk1 is essential to drive cell cycle in the absence of other Cdk (mouse knock out model)

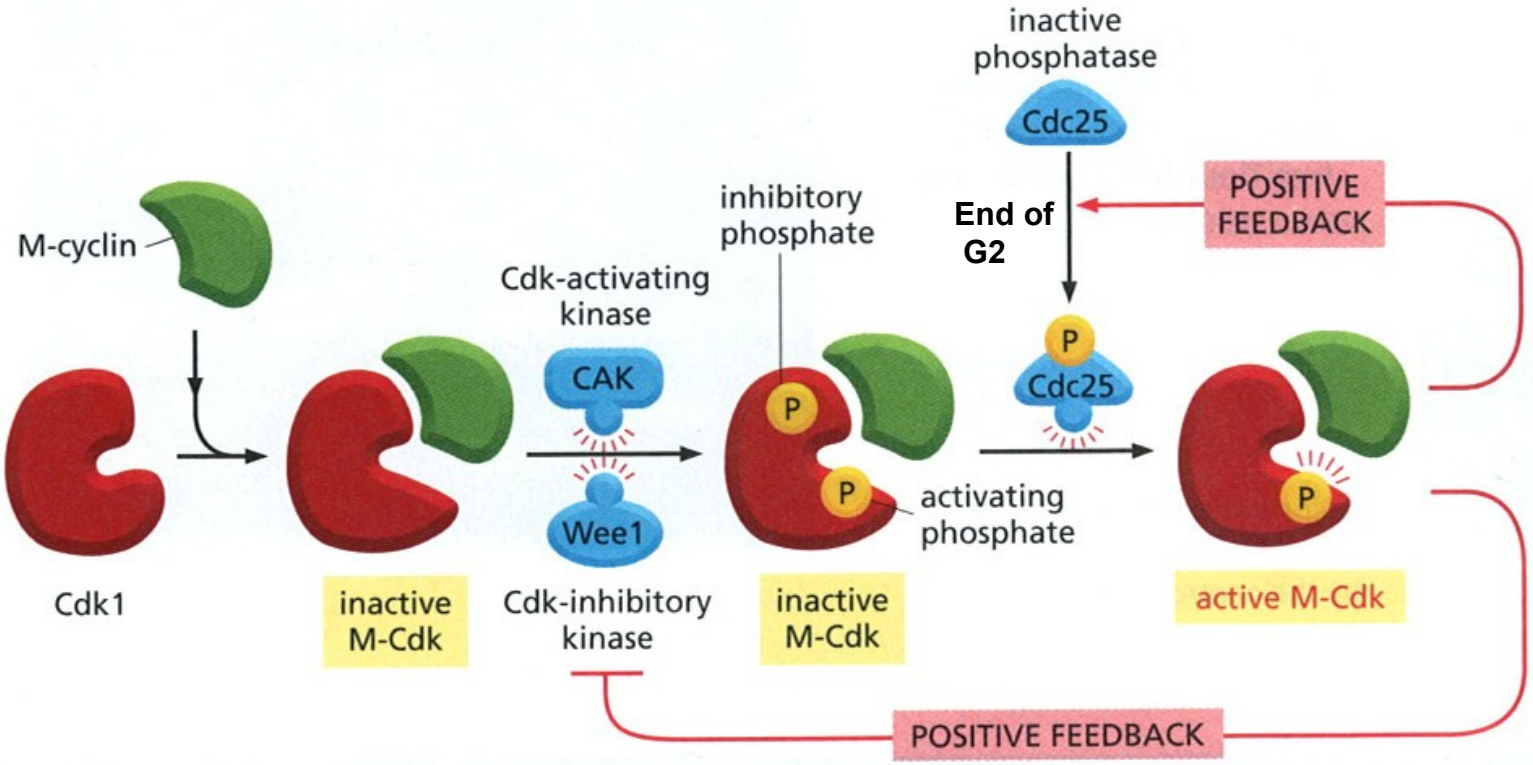
Evolution of cell cycle control

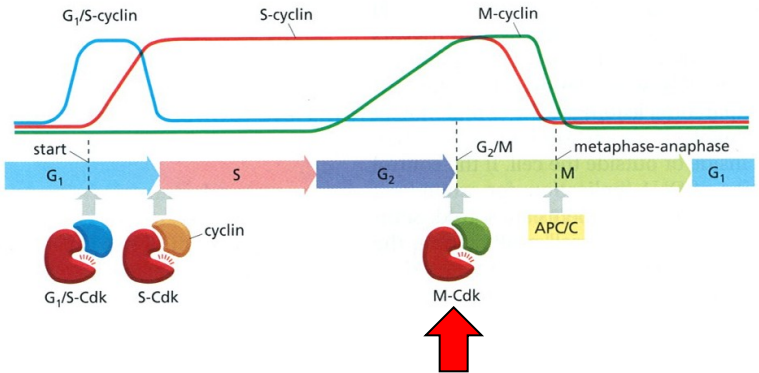


Cell cycle control system is a network of biochemical switches where Cyc/Cdk complexes play a major role



Activation of M-Cdk (cycB/cdk1)

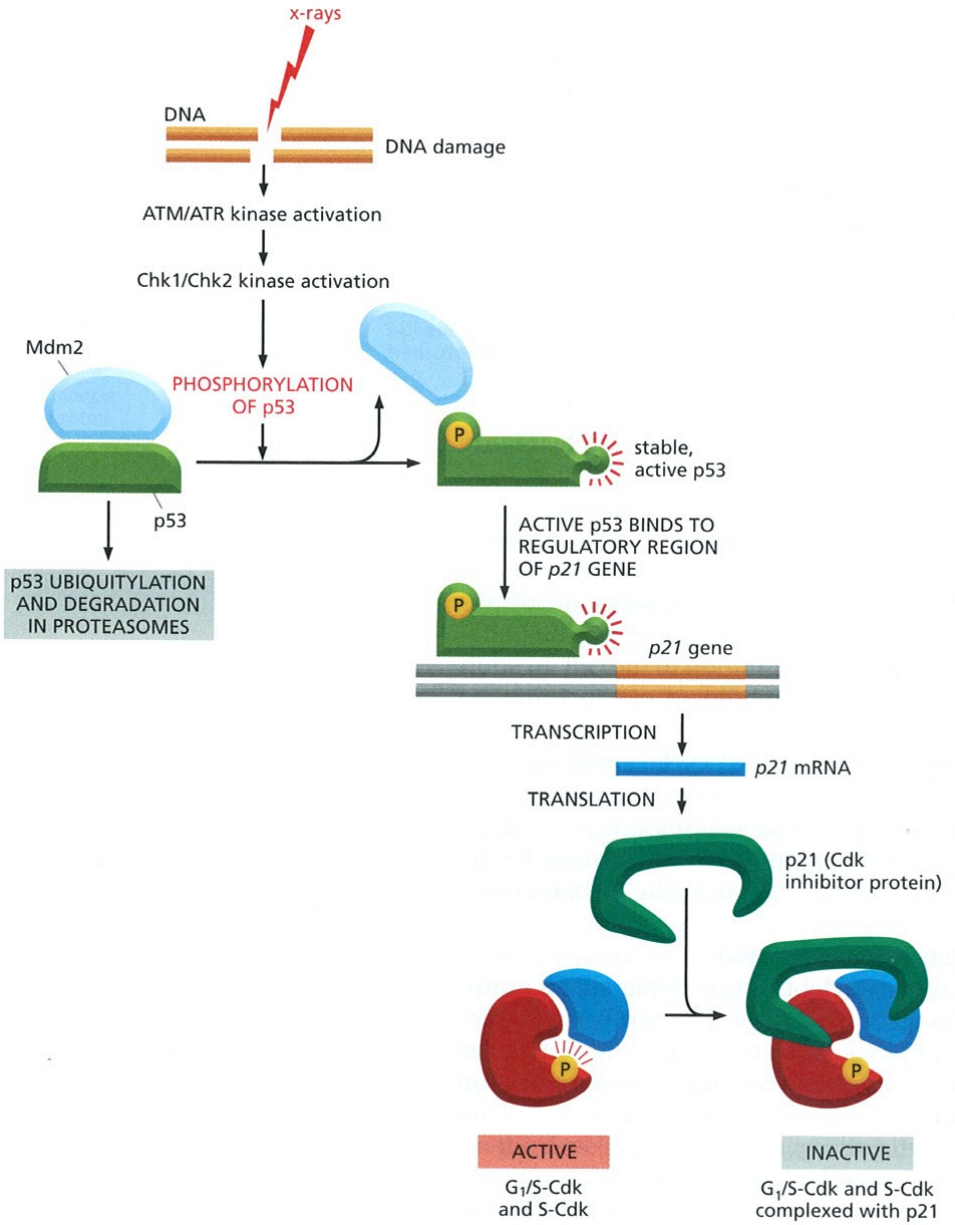


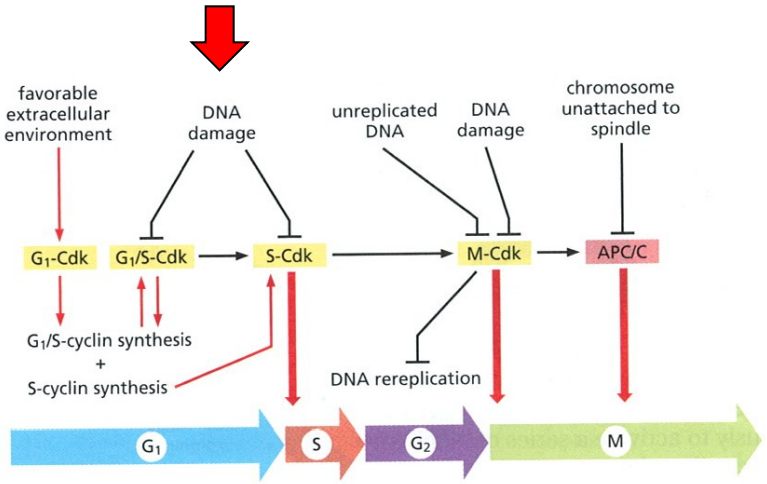


De-phosphorylation activates accumulated M-Cdk at the onset of mitosis

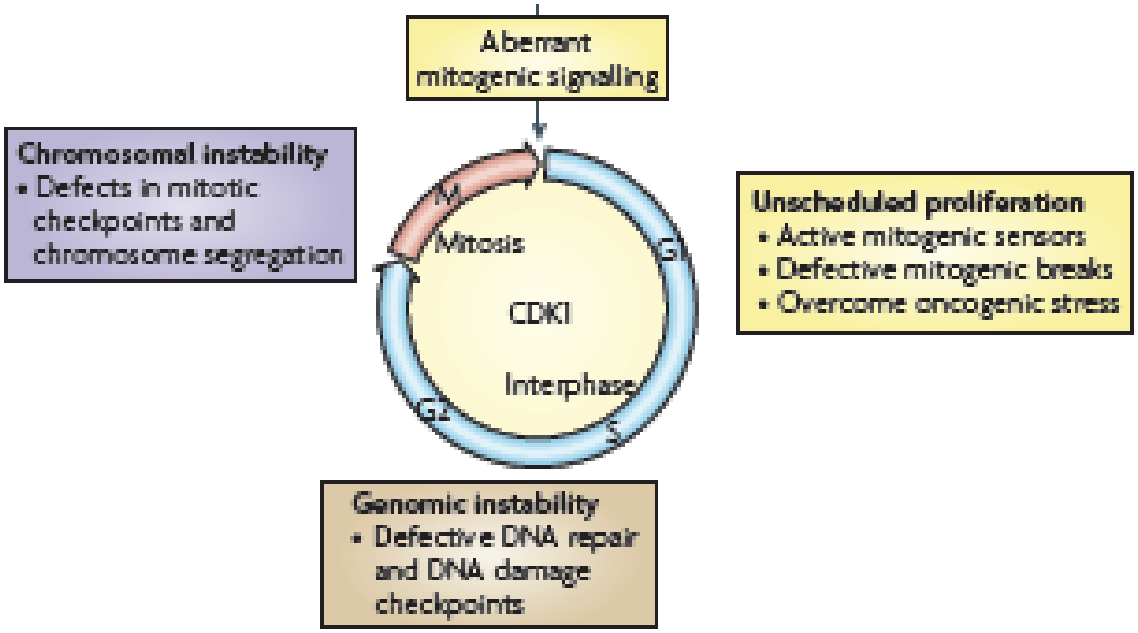
Mechanism of cell cycle arrest in G1 by DNA damage

DNA damage causes transcription of p21, Cdk inhibitory protein, that inhibits G1-S- and S-Cdks, arresting the cell cycle in G1 phase



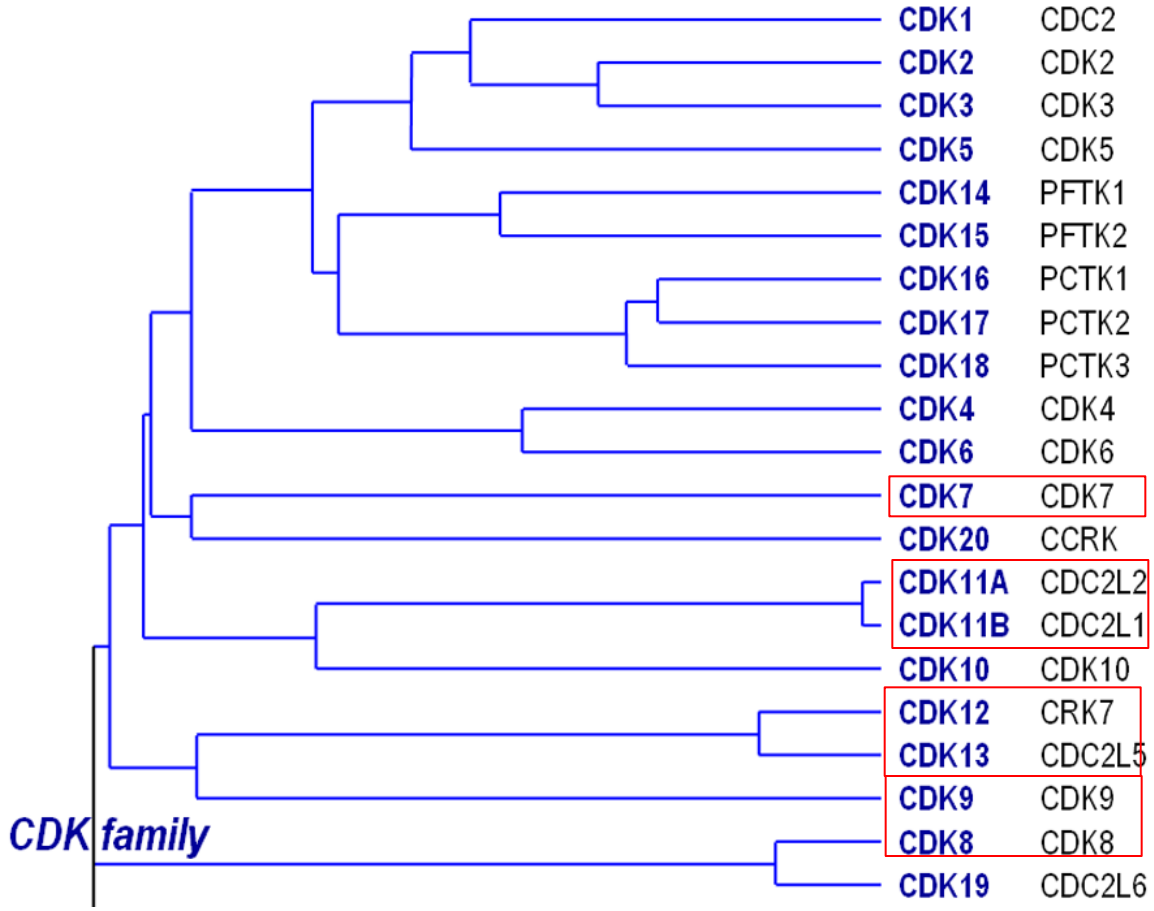


Deregulation of cell cycle and cancer



Cells escape from the proper control of the cell cycle during cancer development:
-Increase in expression and activity of proteins driving cell cycle regulators (Cdks)
-Inactivation of inhibitors of Cdks

Regulation of transcription by Cdks



Transcriptional Cyc/Cdk complexes

Cdk	Other nomenclature	Yeast homolog	Cyclin
Cdk7	CAK	Kin28	CycH [32]
	CAK1		
	STK1		
	MO15		
Cdk8		Srb10	CycC [36]
Cdk9-42 kDa	PITALRE	Bur1 [6]	CycT1 [17,18] CycT2a/b [17,18]
Cdk9-55 kDa			CycT1 [38]
Cdk11-46 kDa			CycL.1 [39]
			CycL.2 [39]
Cdk11-58 kDa			CycL.1 [39]
			CycL.2 [39]
			CycD3 [40]
Cdk11-110 kDa	PITSLRE	Ste20	CycL.1 [39,41]
	CDC2L2		CycL.2 [39,41]
Cdk12	CRKRS CRKS	Ctk1 [6]	CycK [5,6]
	CRK7		
	PITAIRE		
Cdk13	CDC2L5	Ctk1 [6]	CycK [5]
	PITAIRE		

Major differences between Transcription and Cell Cycle Cyc/Cdk complexes

Trancription Cyc/Cdks complexes:

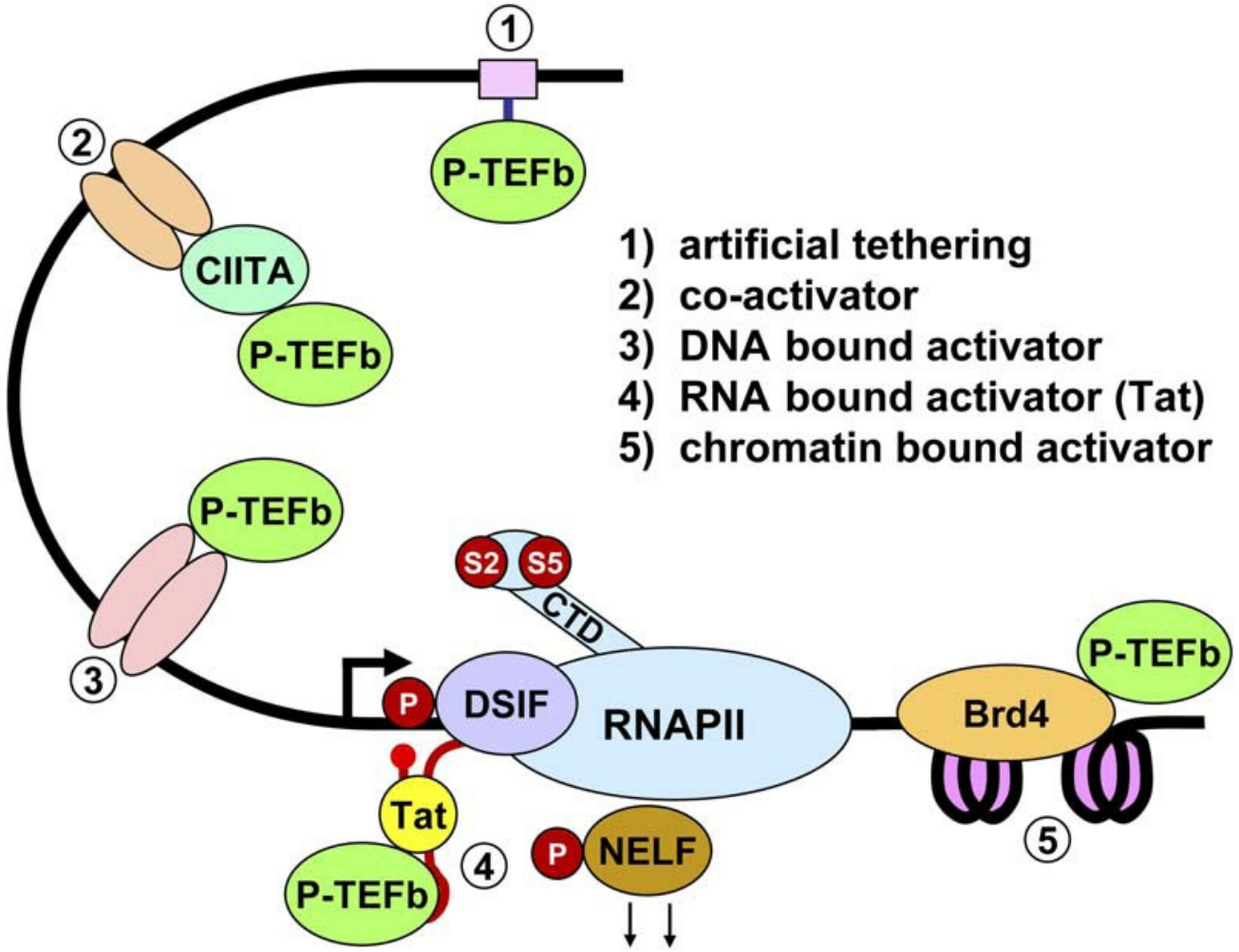
1)Cdk has usually only one Cyclin partner

2)Usually in multi-protein complexes

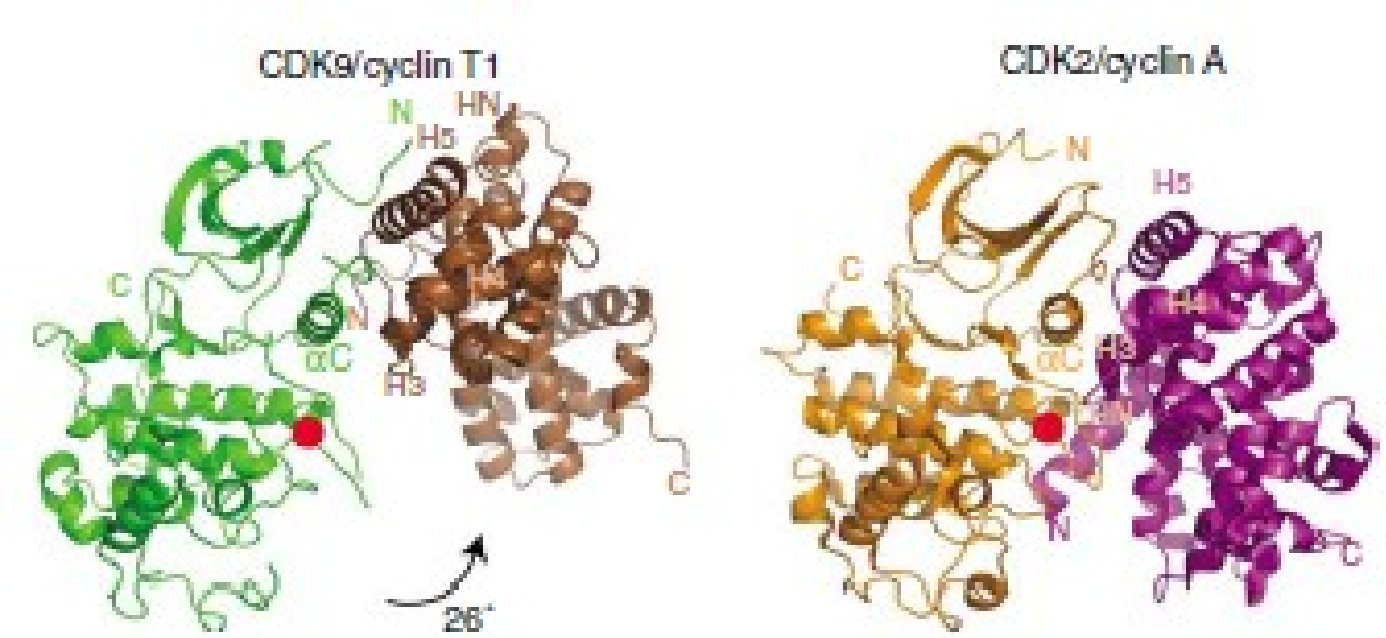
3)The Cyclin levels in cells do not oscilate
(Cdks need to be constantly active for basal transcription)

4)Regulated at the level of recruitment to specific gene

Ad 4) Examples of recruitment of P-TEFb (Cdk9) to genes

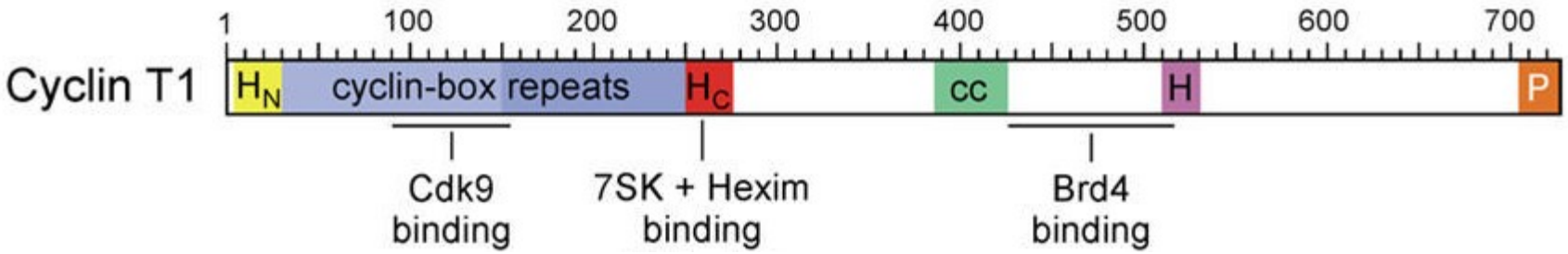


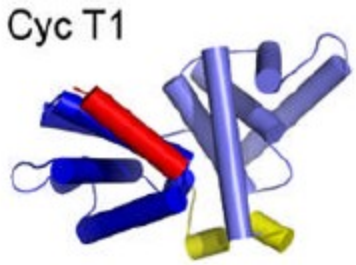
Differences between Cell Cycle and Transcription Cyc/Cdks-structure

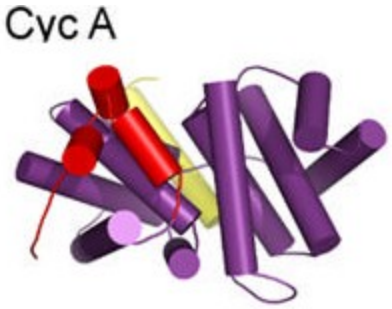


Sparse number of contacts btw Cyc and Cdk in transcription Cyc/Cdk complexes
More contacts in Cell Cycle Cyc/Cdk complexes - important for Cdk activation

Differences between Cell Cycle and Transcription Cyc/Cdks- Cyclin structure







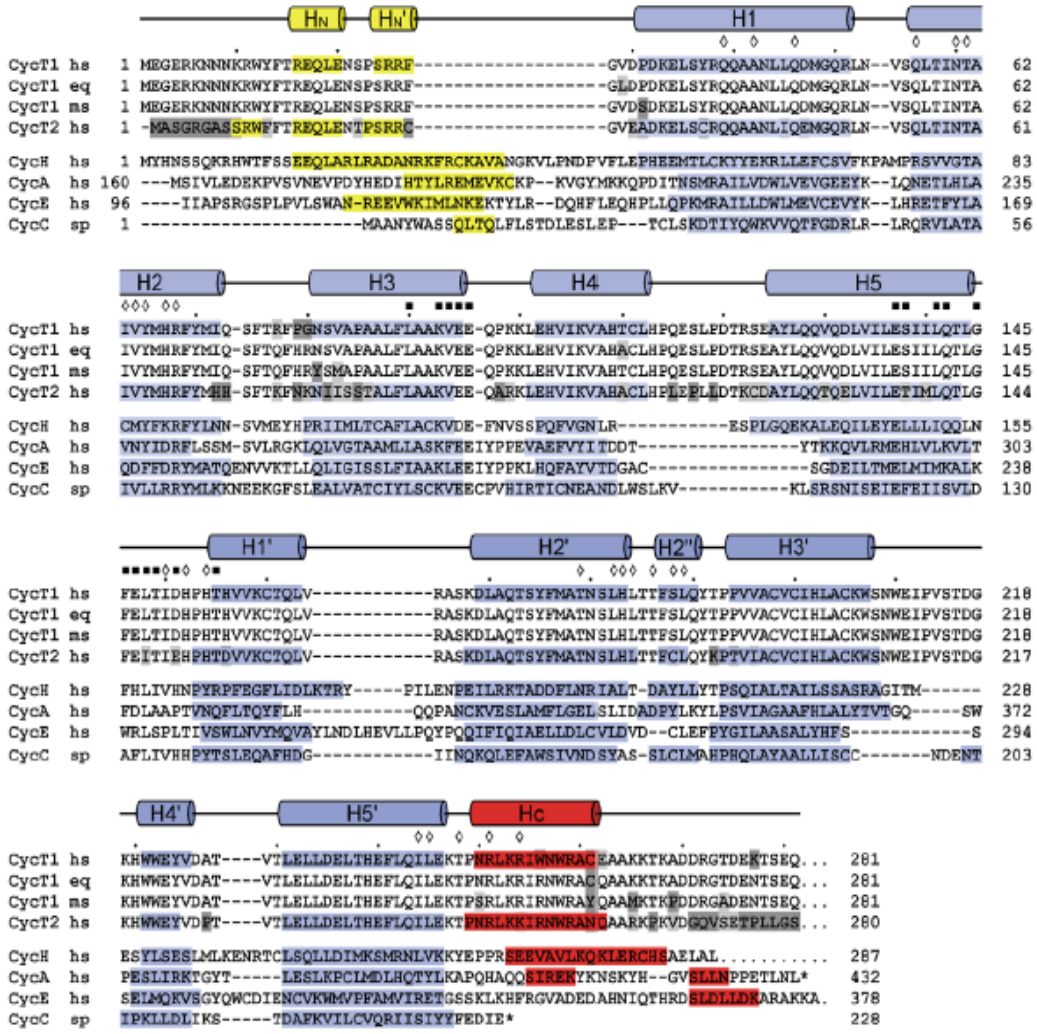
All Cyclins have 2 canonical cyclin-boxes responsible for Cdk binding

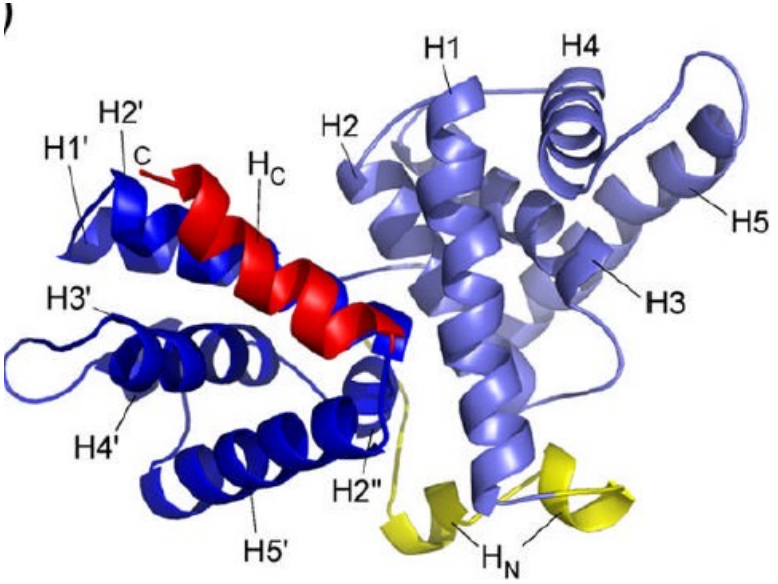
Each cyclin-box consists of 5 helixes

The cyclin-boxes conserved in all Cyclins

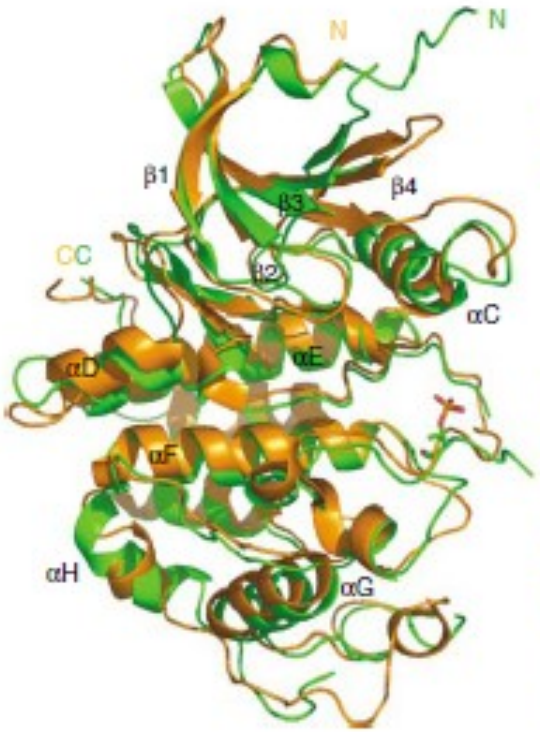
Cell Cycle and Transcription Cyclins differ significantly in sequence and structure outside of the cyclin boxes (binding to other proteins)

Differences between Cell Cycle and Transcription Cyc/Cdks- Cyclin structure

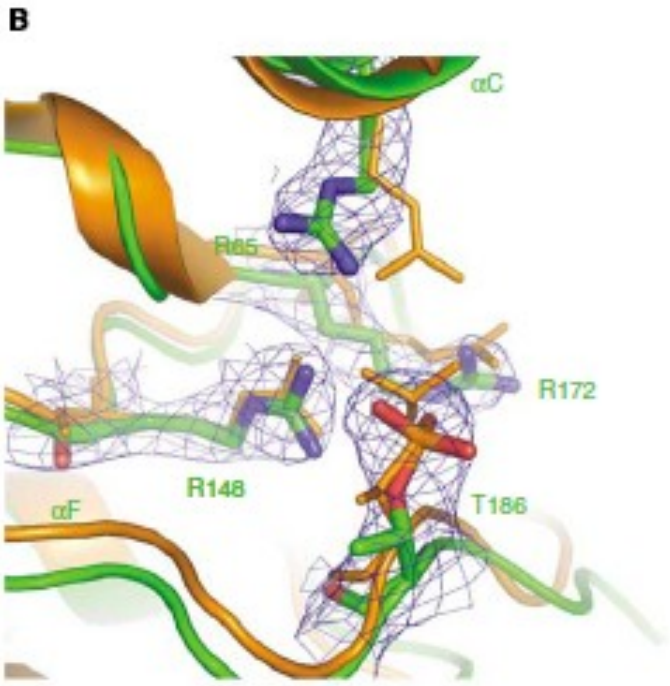




Comparison of Cdk9 and Cdk2



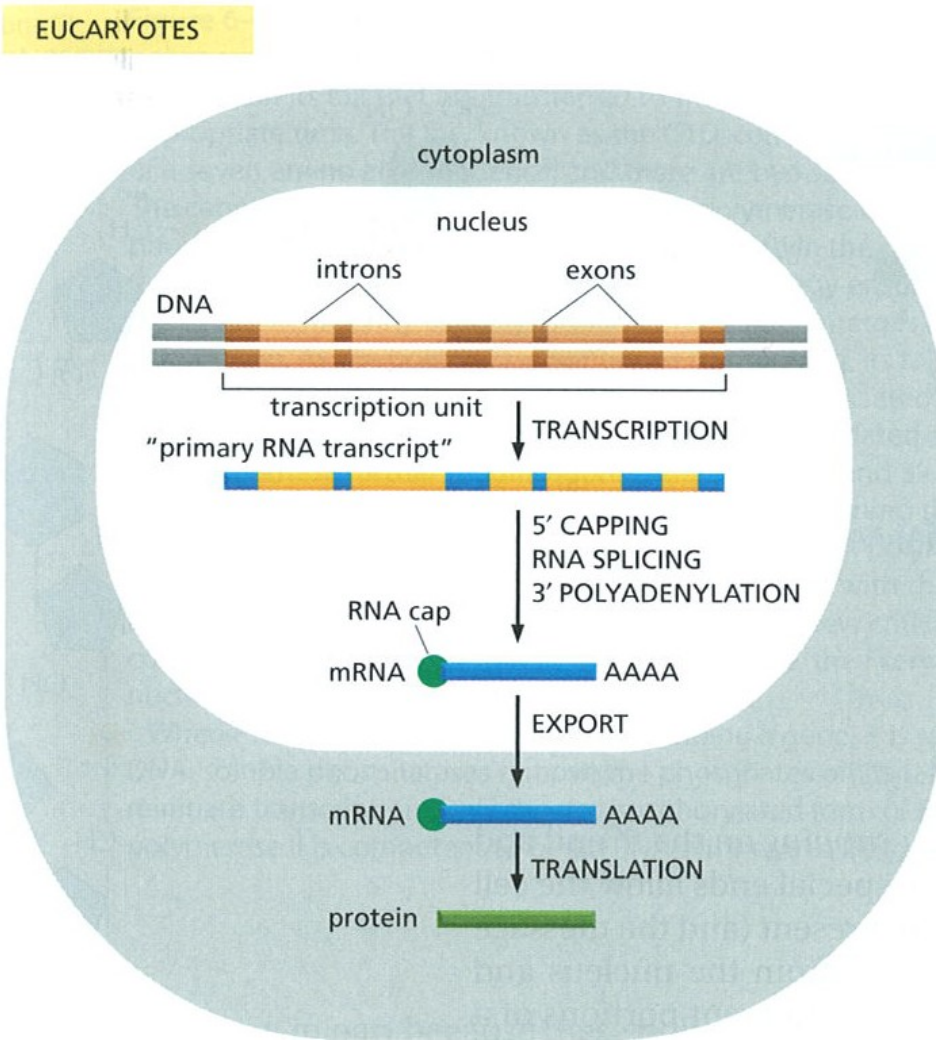
Cdk9 (green) /Cdk2 (orange)



T-loop (T186/T180)

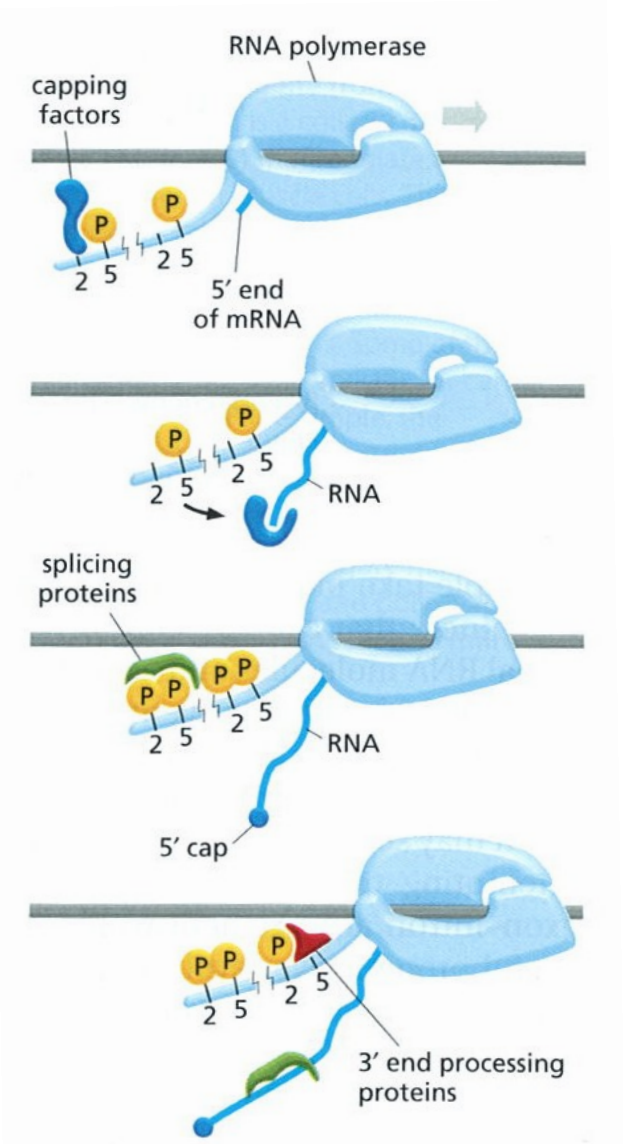
Structures very similar, sequence similarity 40%

Transcription



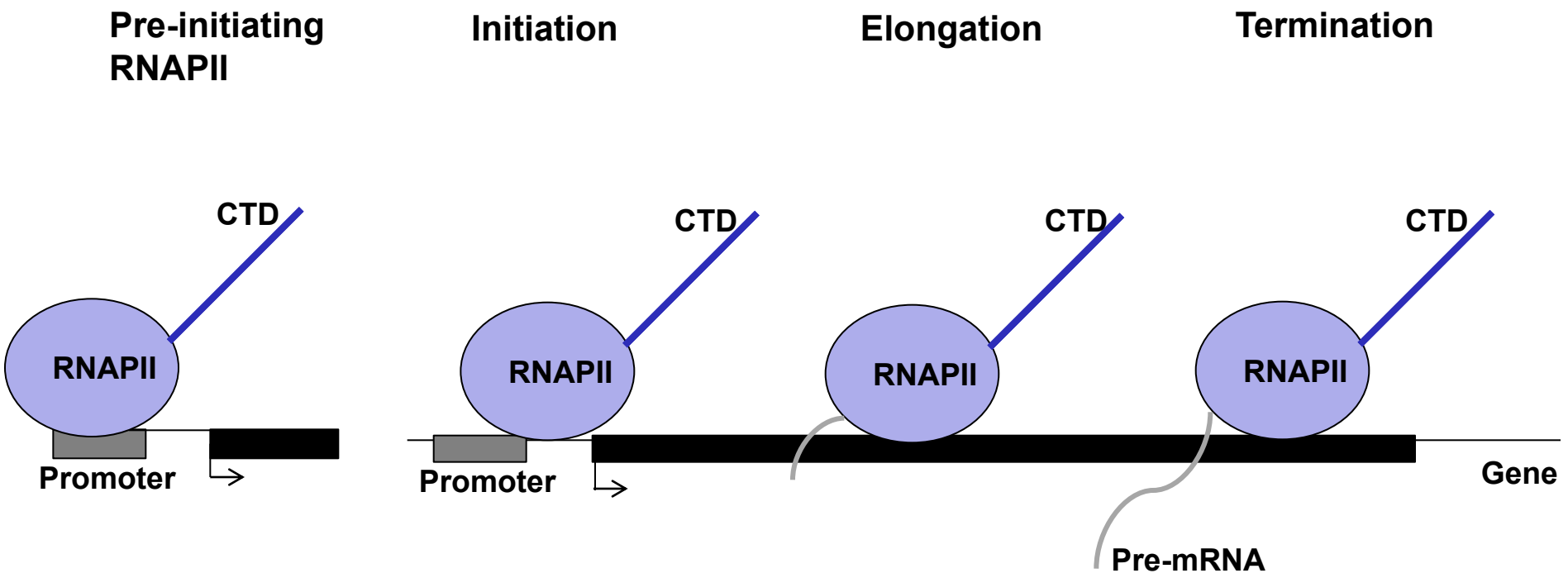
Transcription- synthesis of RNA from DNA template

Transcription in eukaryotes is tightly linked to co-transcriptional mRNA processing



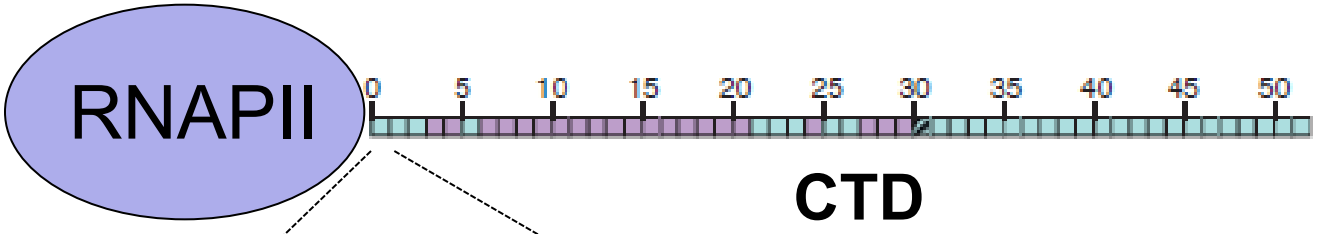
The co-transcriptional mRNA processing (capping, splicing, 3` prime end processing)

Transcription of protein-coding genes by RNA polymerase II (RNAPII)



C-terminal domain (CTD) of RNAPII plays a crucial role in regulation of transcription and co-transcriptional mRNA-processing

CTD consists of 52 repeats of heptapeptide YSPTSPS in which individual amino acids get phosphorylated to form a "CTD code"

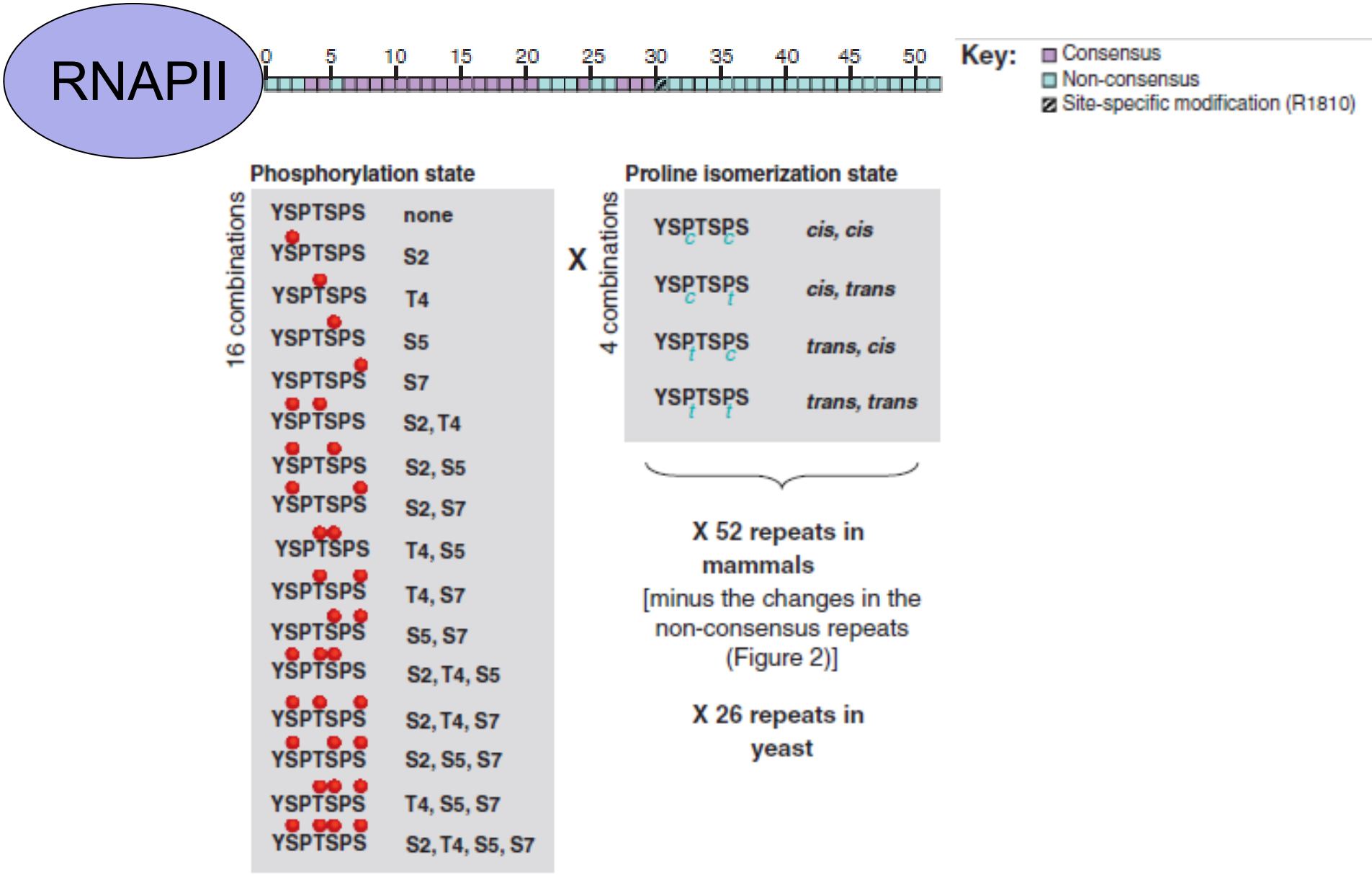


P P iso P P iso P

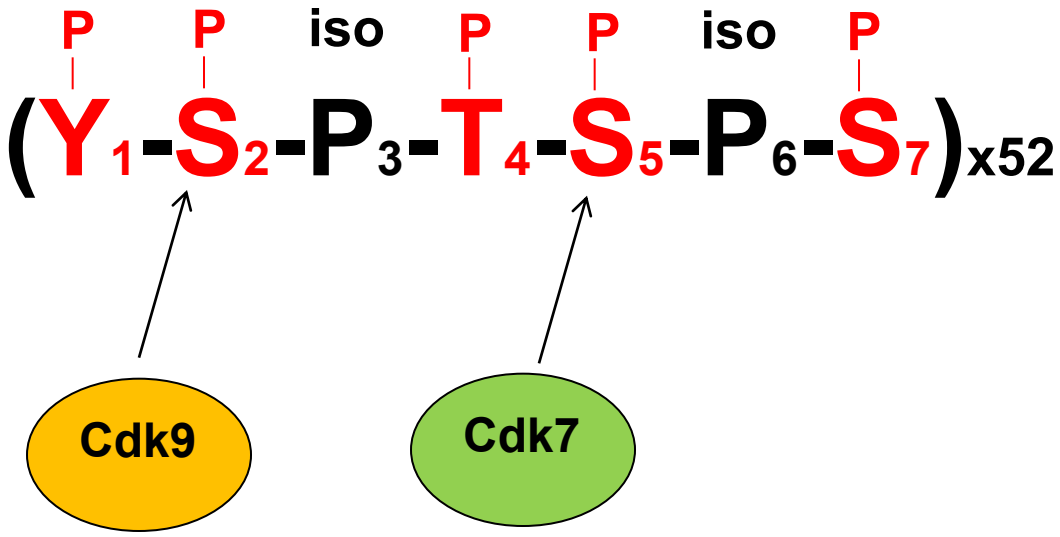
$(Y_1\text{-}S_2\text{-}P_3\text{-}T_4\text{-}S_5\text{-}P_6\text{-}S_7)x52$

-52 repeats in humans (21 consensus, 31 non-consensus)
-26 repeats in yeast
-evolutionary conserved-important!

Human “CTD code”

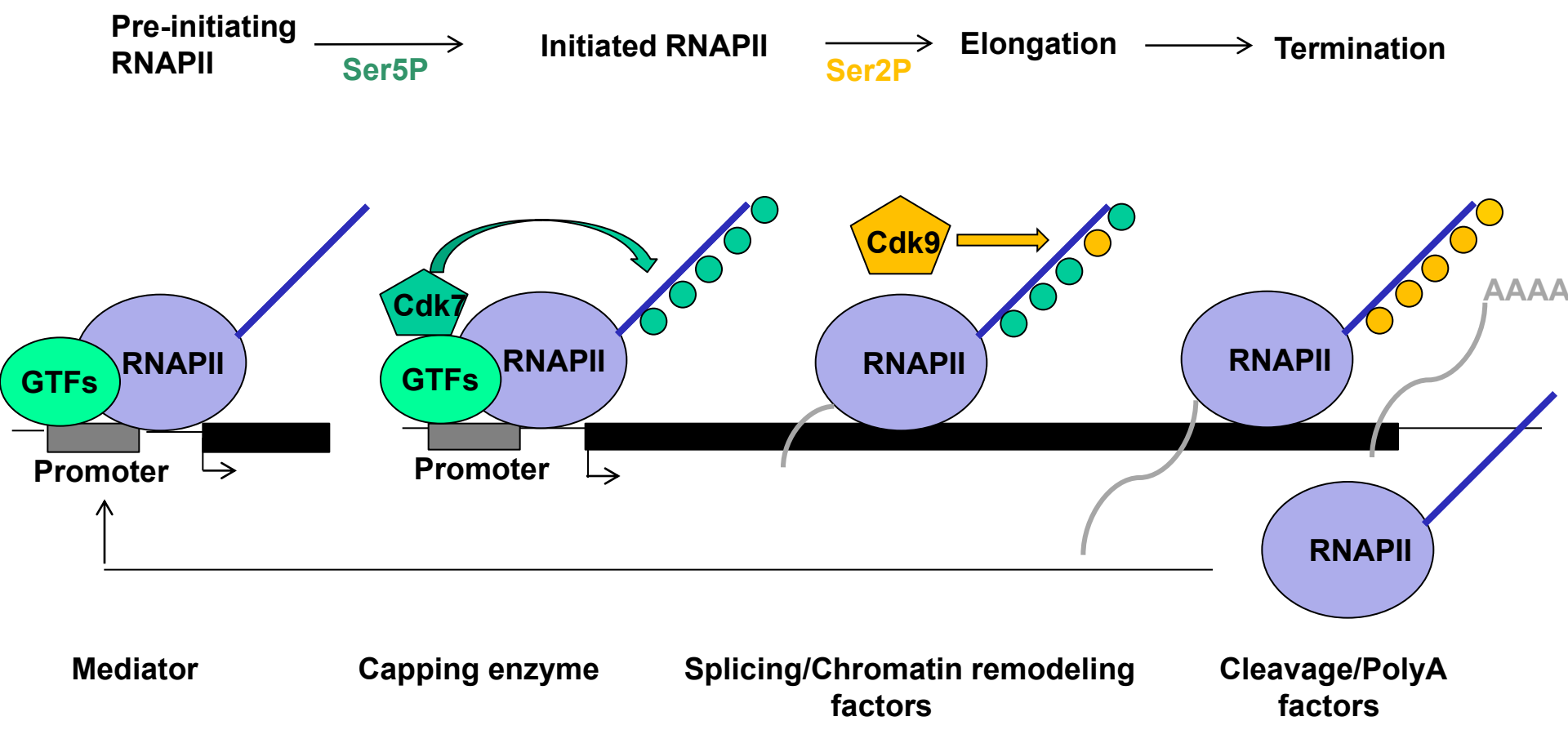


Repeats of the CTD get phosphorylated by the Cdks

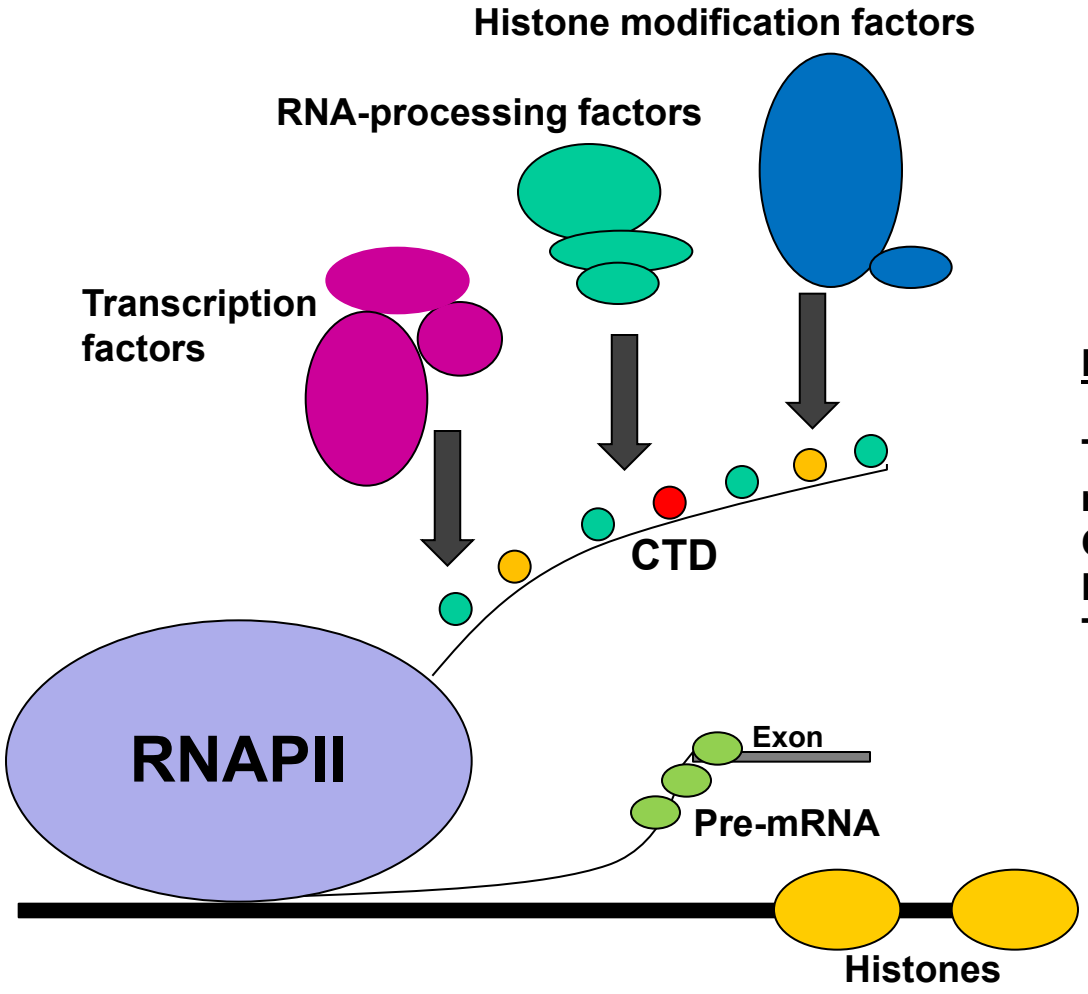


Cdk9 phosphorylates Serine (Ser) in the position 2
Cdk7 phosphorylates Serine (Ser) in the position 5

For the regulation of transcription cycle the phosphorylations of the CTD by the Cyc/Cdks are essential



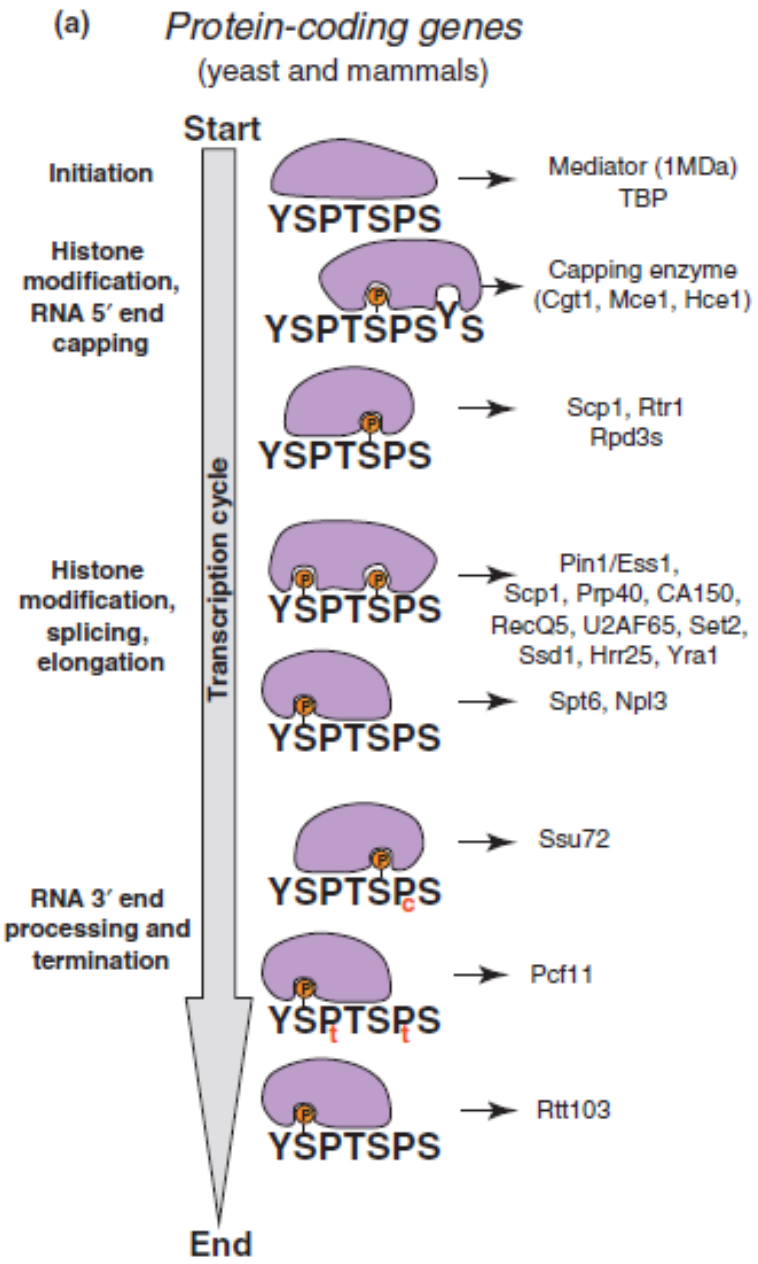
Modified CTD is a binding platform for transcription factors, RNA-processing factors and histone modification factors (code readers)



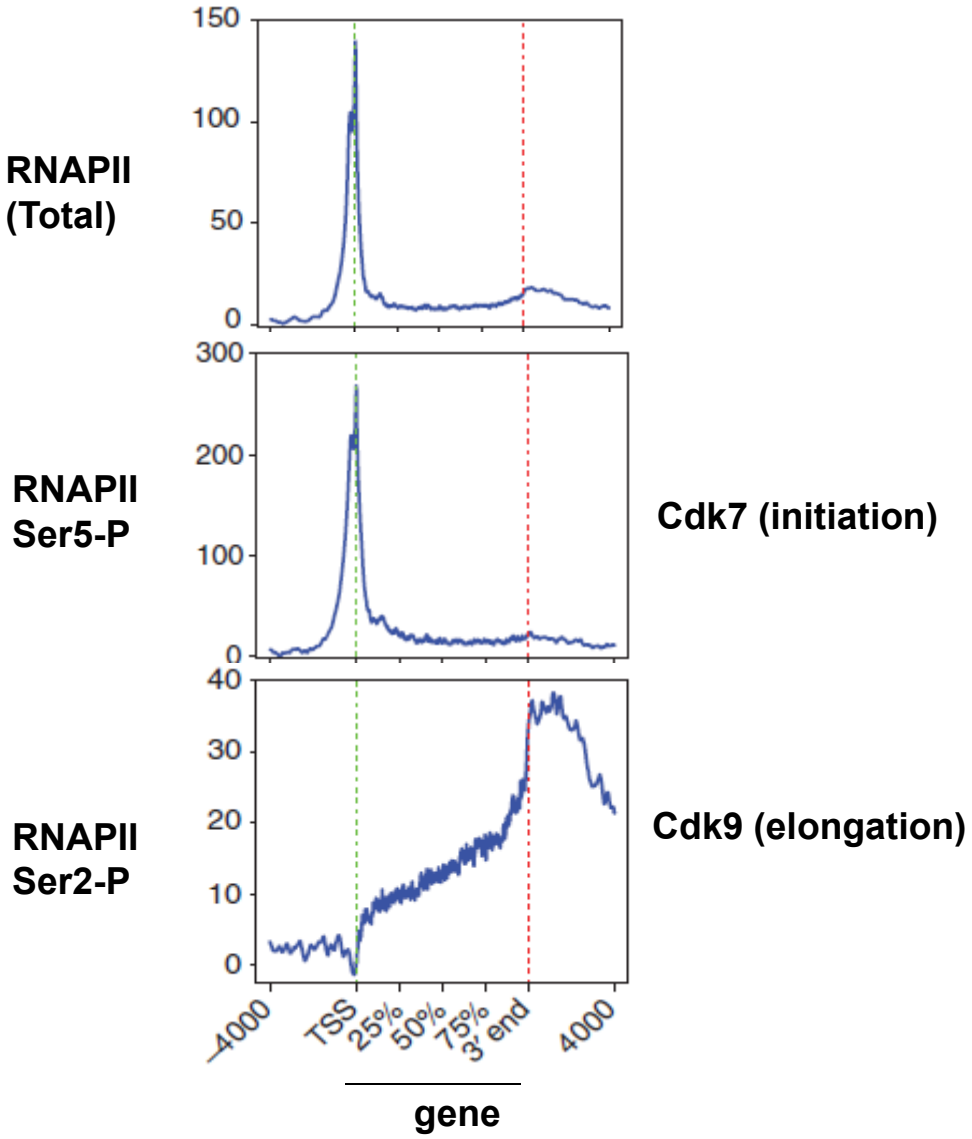
Phosphorylation of the CTD mediates:

Transcription
mRNA-processing
Chromatin modifications
RNA export
Transcription-coupled genome stability

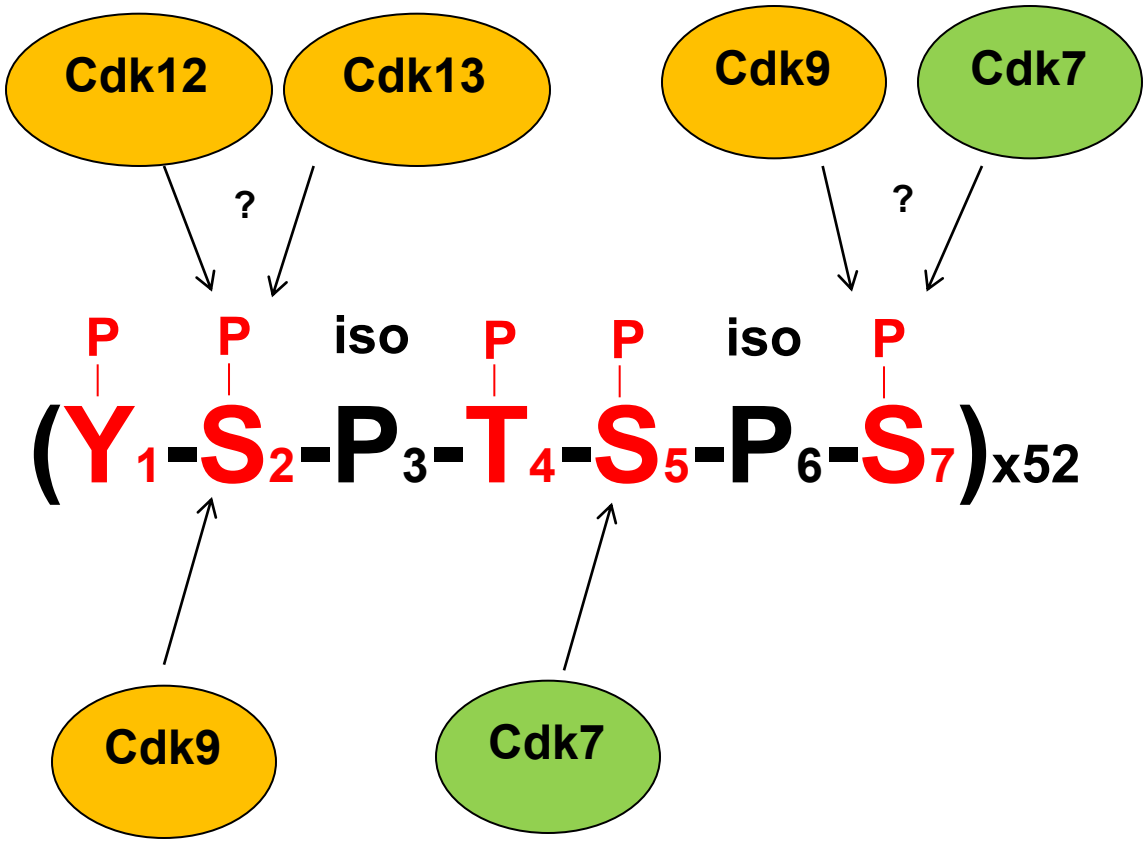
CTD code readers



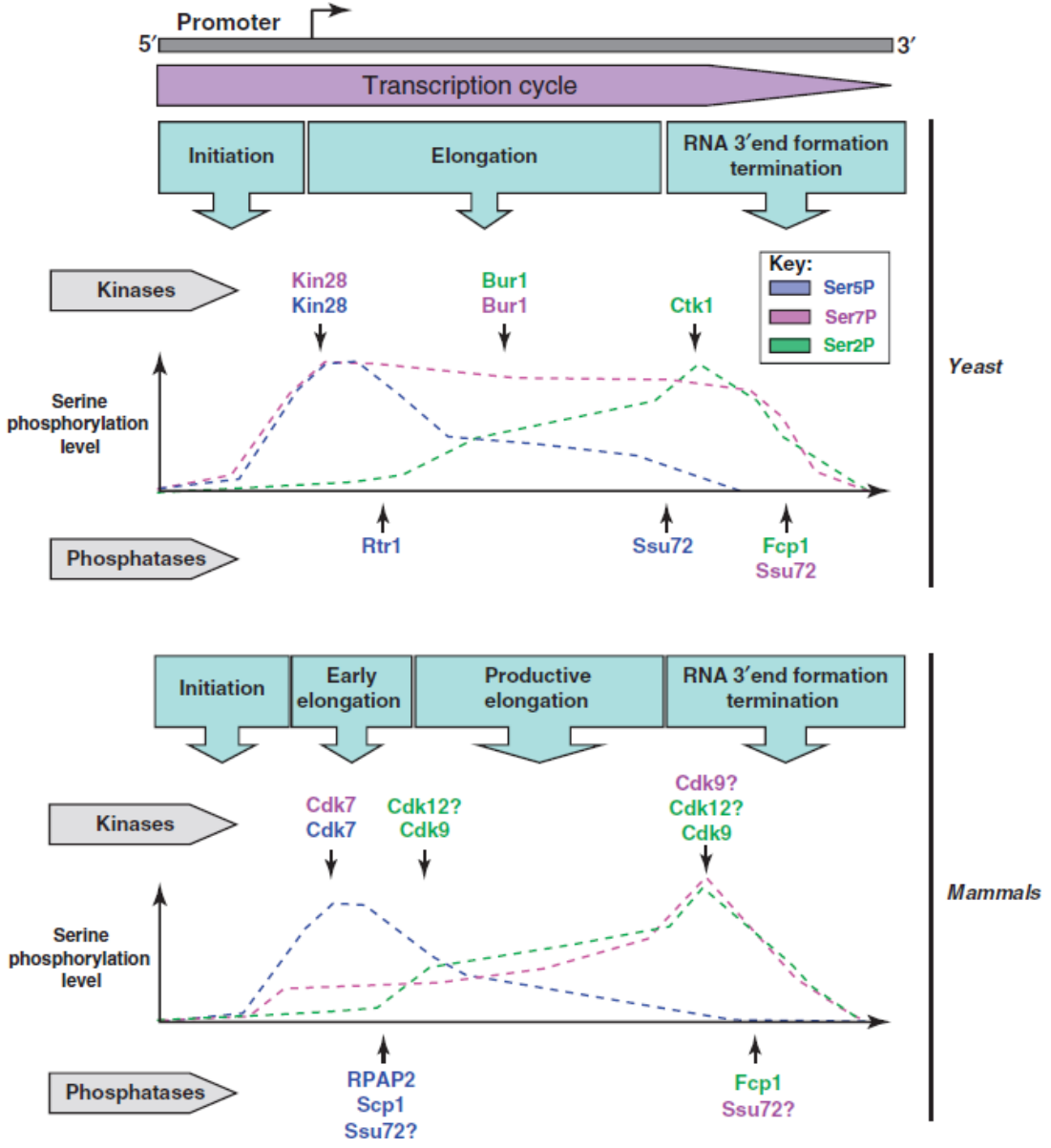
Distribution of phosphorylated Serine 5 and Serine 2 in the CTD of RNAPII along the human protein coding genes



Roles of new Cdks in the CTD modification (CTD code)



Cdks and their roles in transcriptional cycle of yeast and human



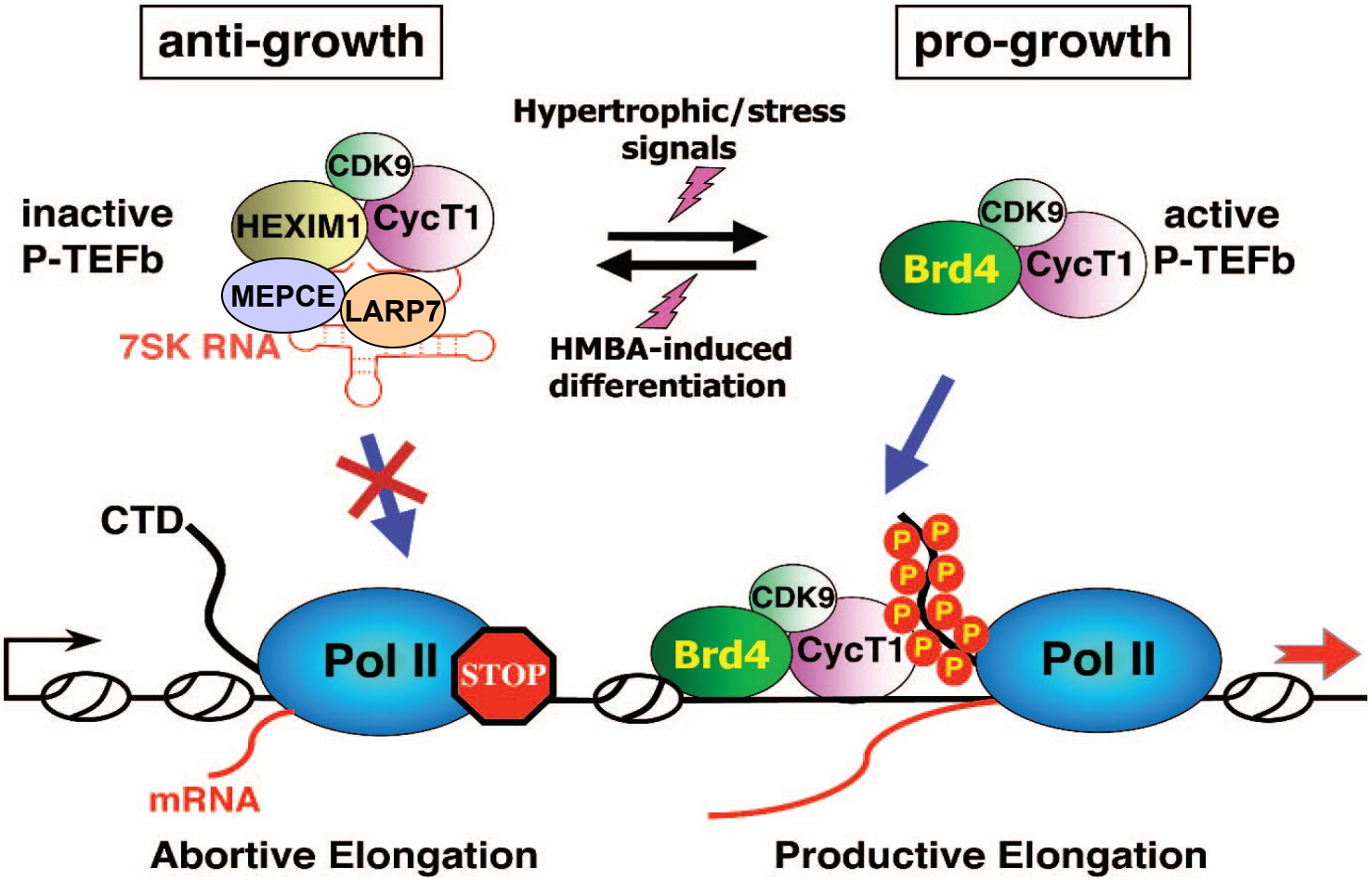
Deregulation of transcription by Cdks leads to the onset of human diseases

-Cancer - aberrant kinase activity of Cdk9 , Cdk12

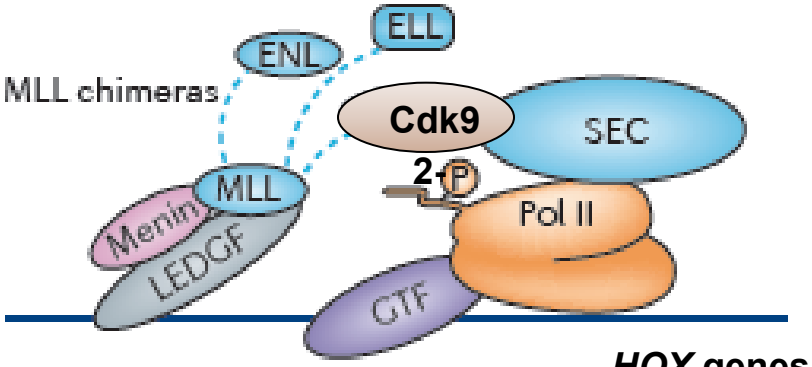
defective transcriptional elongation, mRNA processing

-HIV transcription- HIV Tat protein "steals" Cdk9 from its cellular complex to transcribe HIV genome

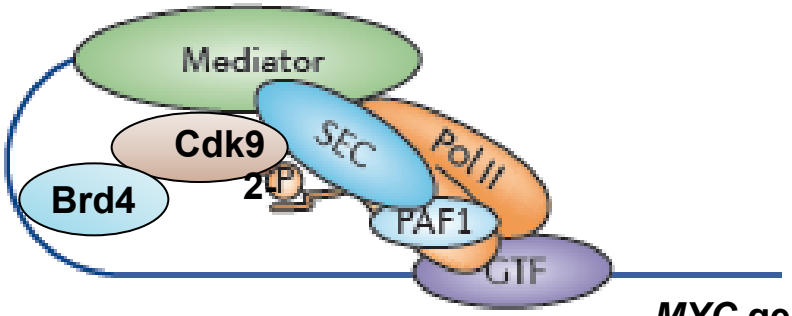
Cdk9 is recruited to most of RNAPII promoters and is present in catalytically active (small) and inactive (large) complexes and regulates transcriptional elongation



Cdk9-dependent transcriptional elongation is a highly regulated process and its deregulation can lead to the onset of cancer



***HOX* genes**



***MYC* gene**

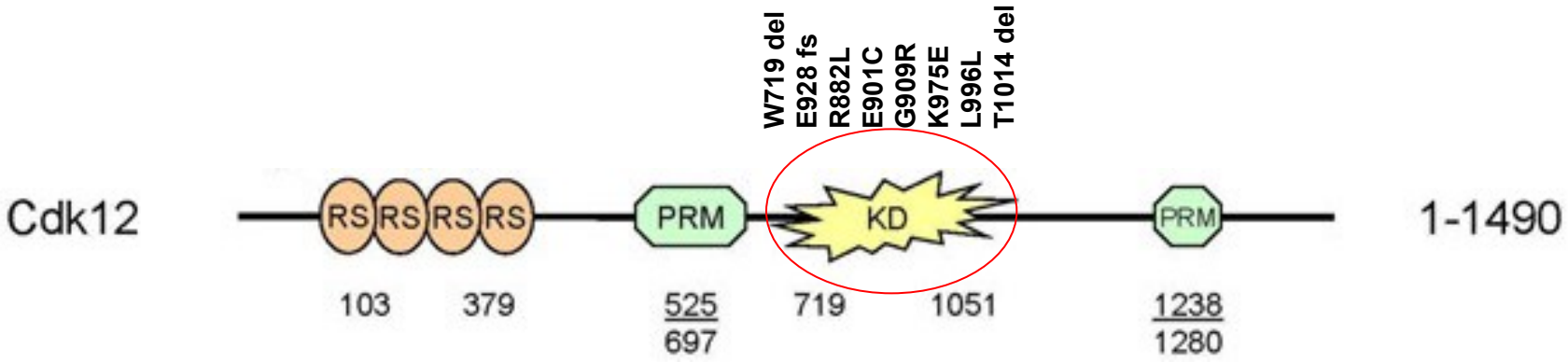
Mixed Lineage Leukemia (MLL)

Abnormal fusion of MLL protein with Cdk9-containing complexes leads to aberrant elongation of *Hox* genes in leukemic cells

Acute Myeloid Leukemia (AML)

Expression of *Myc* gene regulated at the level of Cdk9-dependent transcriptional elongation in this Myc-dependent cancer.

Cdk12 is one of the most often mutated genes in ovarian carcinoma

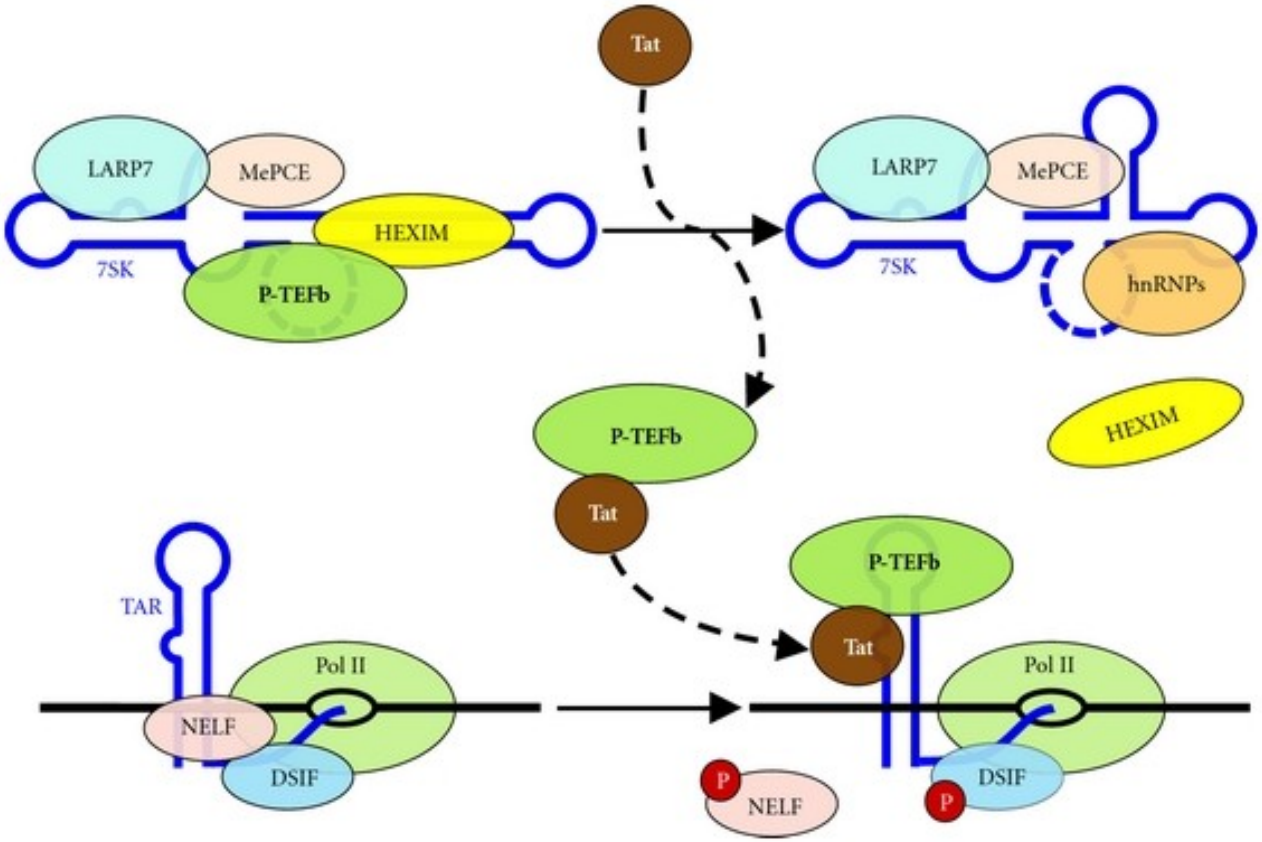


KD=kinase domain

The mutations probably lead to the aberrant kinase activity and defective transcriptional elongation and/or mRNA processing of certain genes

Cdk12 proposed to be a novel tumor suppressor

HIV transcription is dependent on the Cdk9 (P-TEFb) protein



HIV Tat protein "steals" Cdk9 from its complex with inhibitory Hexim1/7SK snRNA; resulting Tat/Cdk9 complex binds to HIV -TAR RNA element and drives HIV transcription in human cells