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

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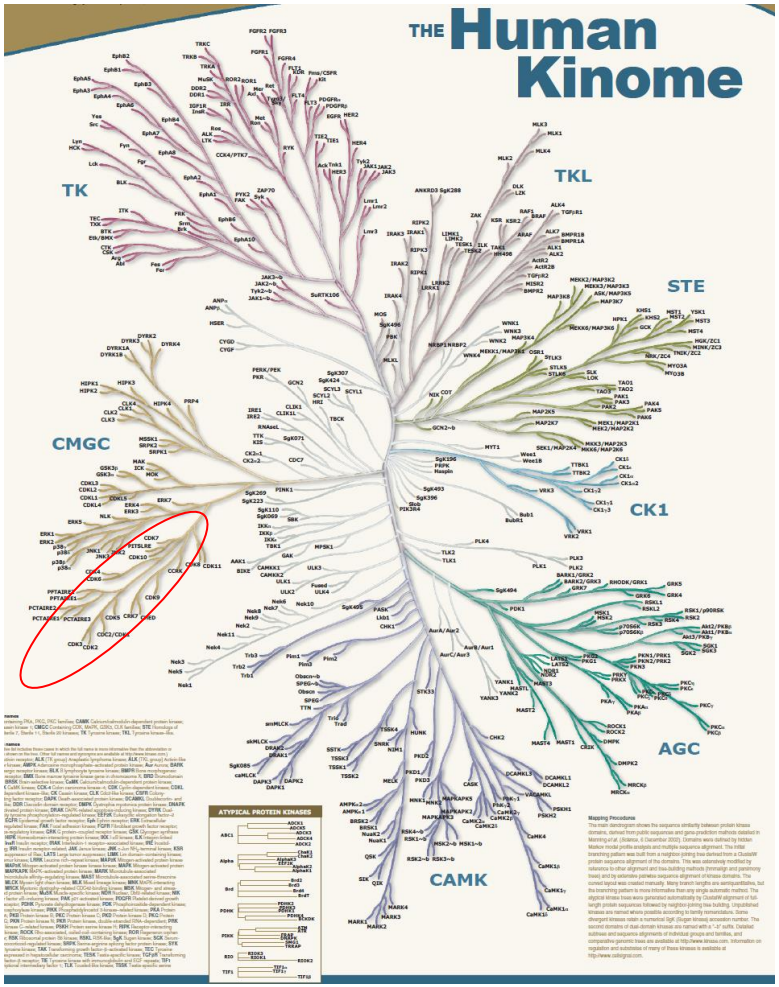
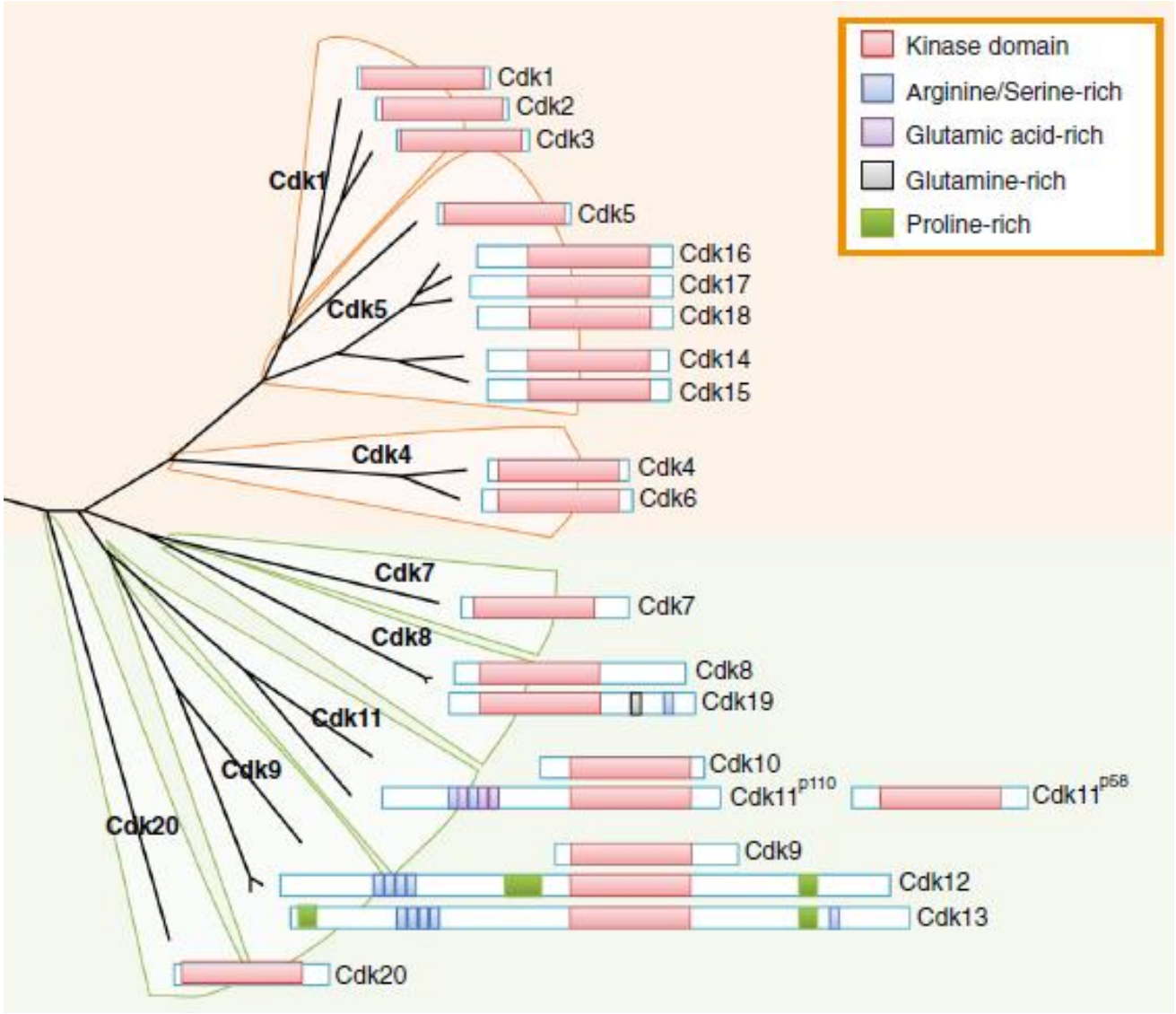
Human kinases



Kinases=proteins that phosphorylate other proteins and direct basic functions in cells

= group of Cyclin-dependent kinases (Cdks)

Cyclin-dependent kinases (Cdks)



Cyclin-dependent kinases (Cdks)

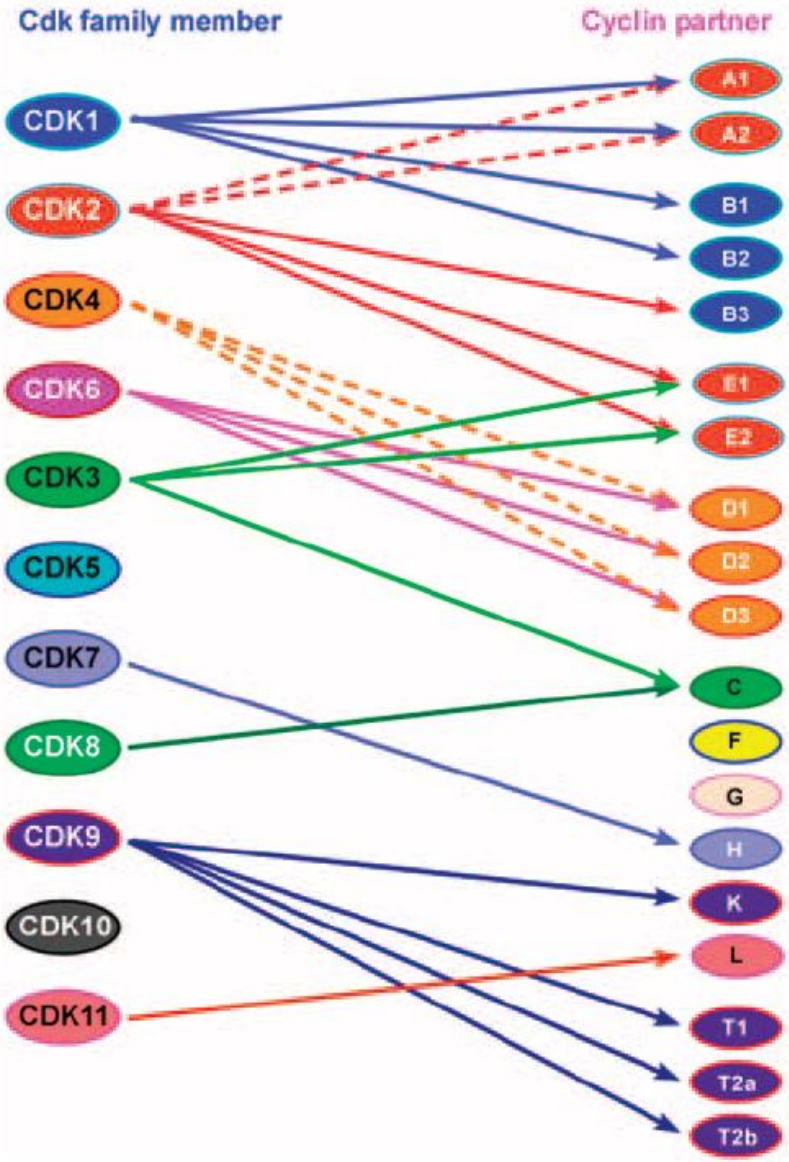
Protein complexes that compose of 1) <u>Kinase subunit</u>
2) <u>Cyclin subunit</u>

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

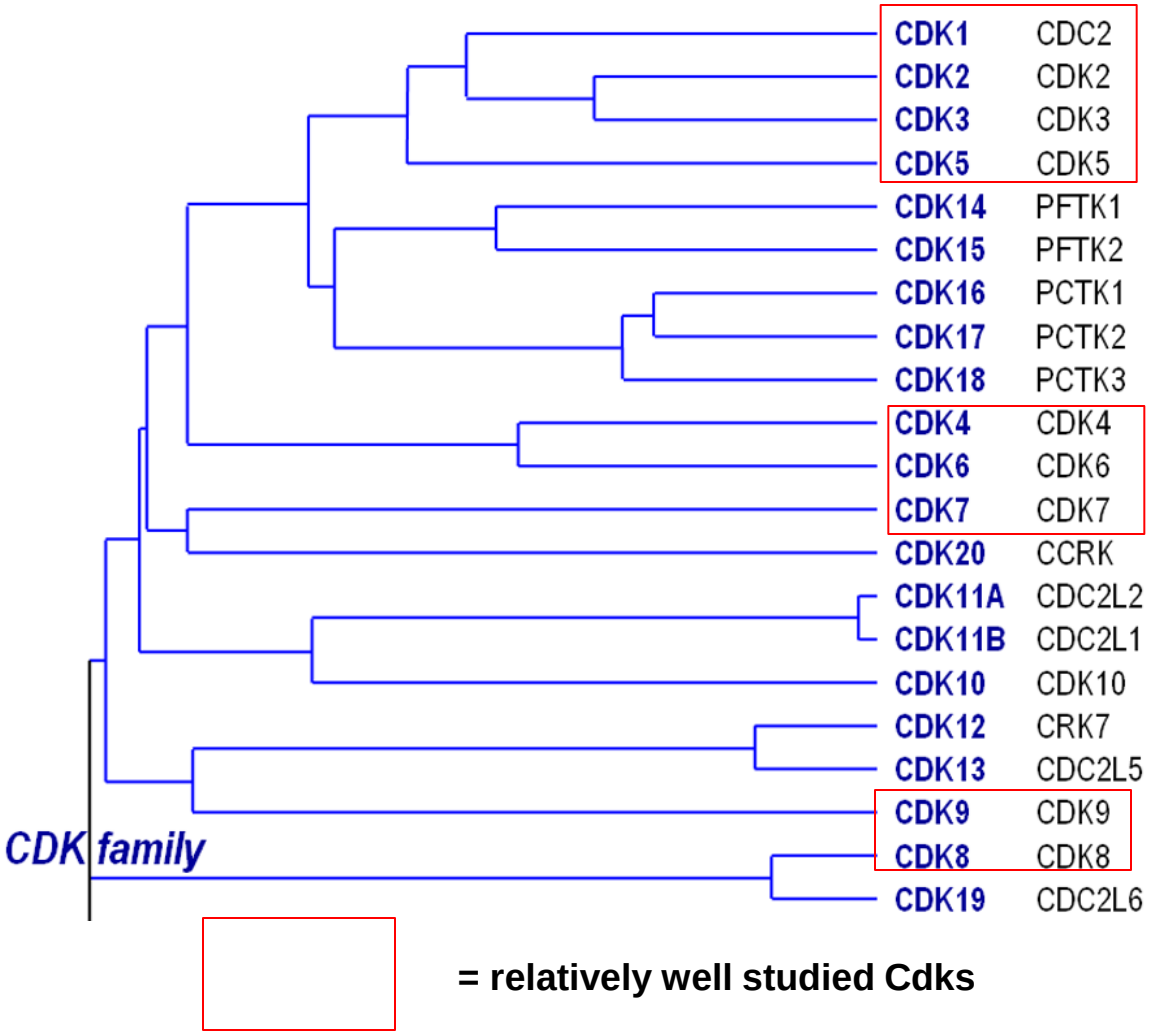
Sequence preference motif: S/T-P-X-K/R

<u>Both subunits needed</u> for the kinase activity of the complex

Most Cdks usually have at least one Cyclin partner



In humans there are at least 20 genes encoding Cdks however only about half of the Cdks are sufficiently studied



Human cell has 20 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)

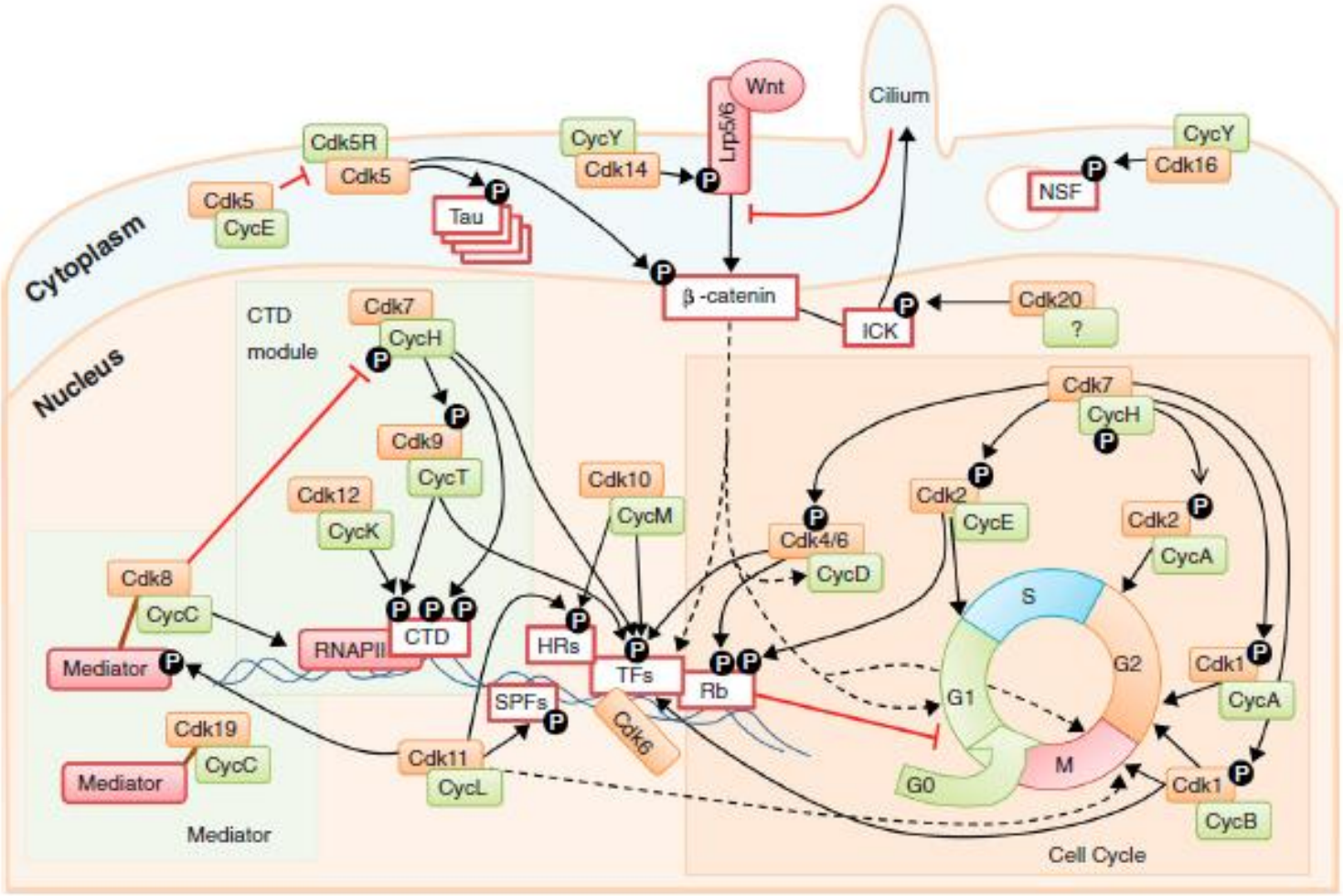
Other functions:

- 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:

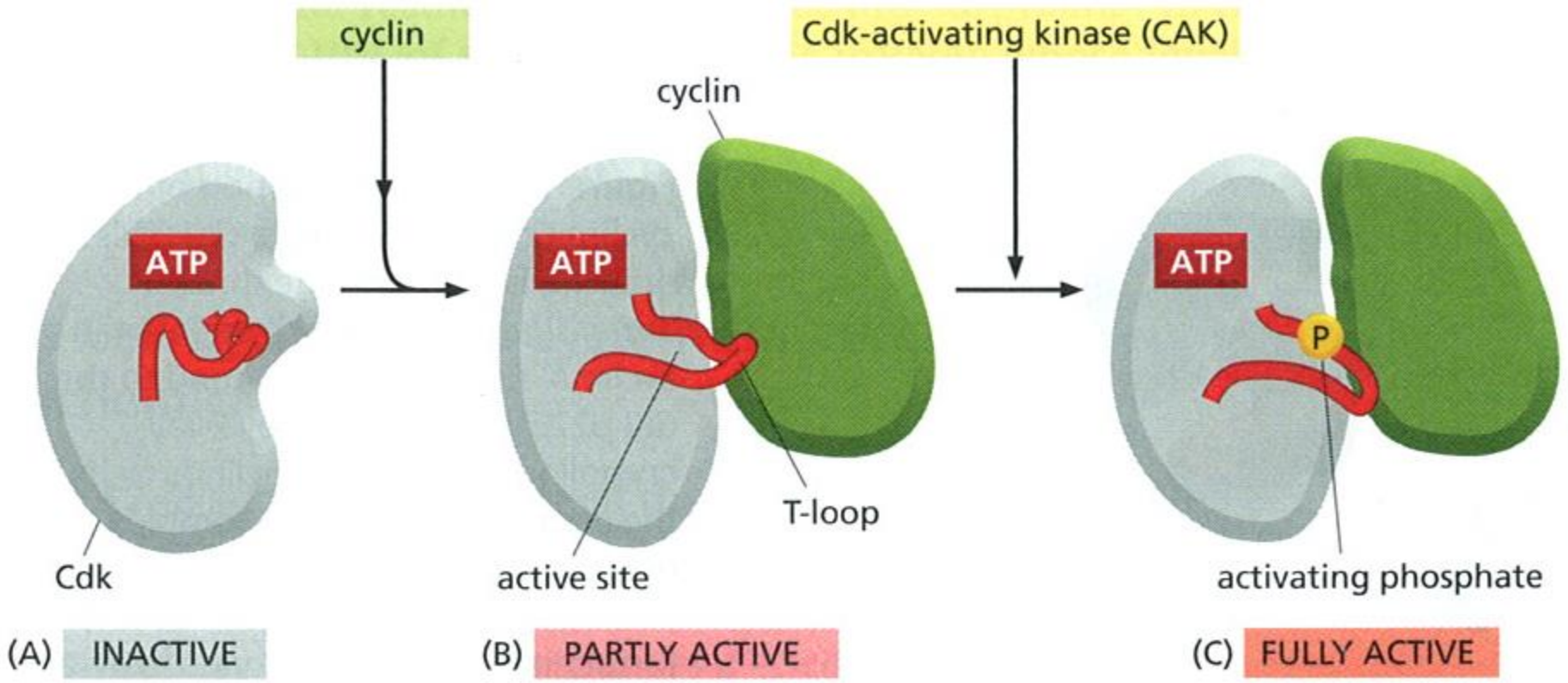
-Association of Cdk with various Cyclin subunits
-Phosphorylation of threonine in the "T-loop" of Cdk
-Degradation of Cdk inhibitor proteins by ubiqitination and proteolysis

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 including small nuclear (sn)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

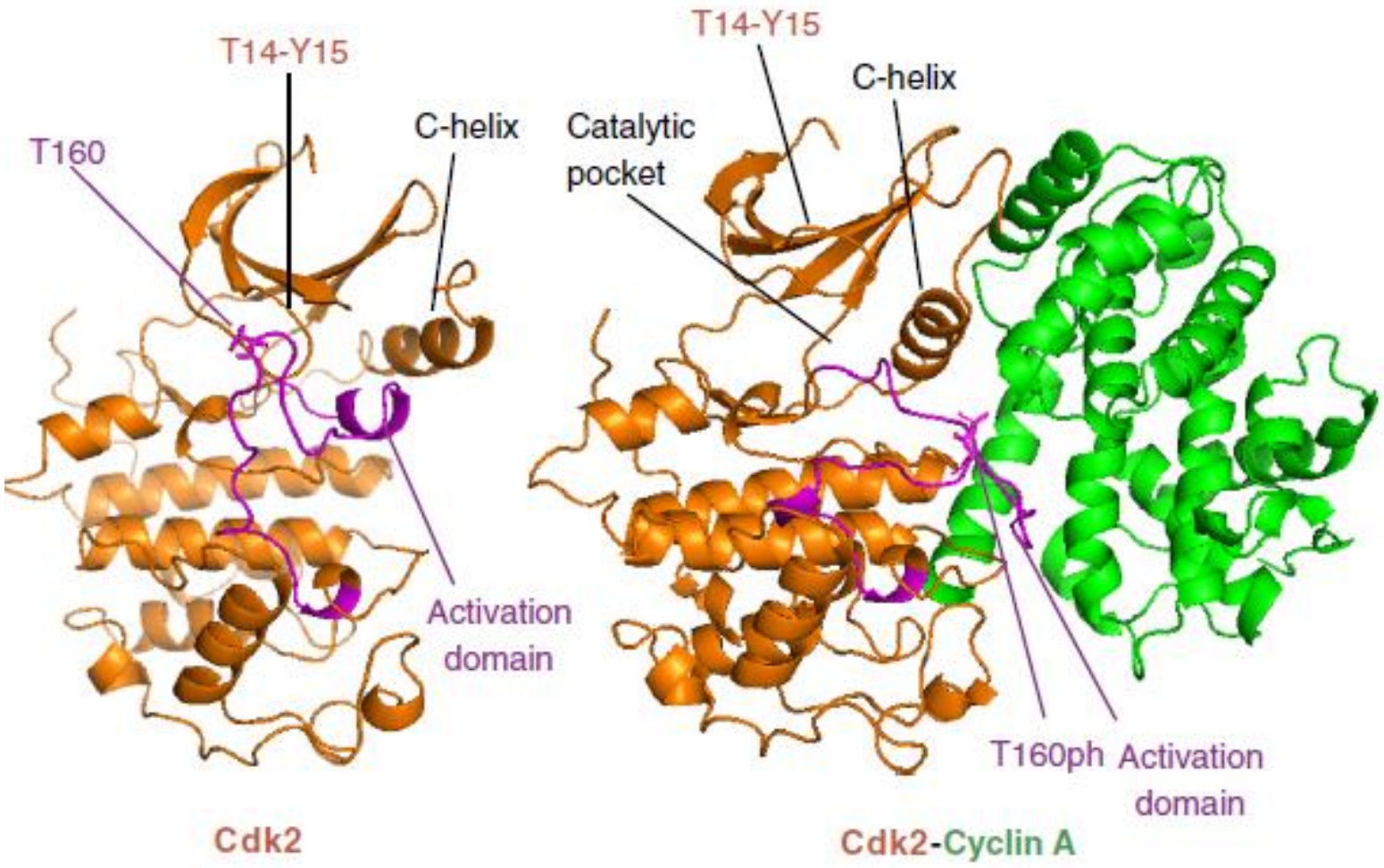


T-loop blocks active site (active site=ATP binding site)

T-loop moves out of the active site

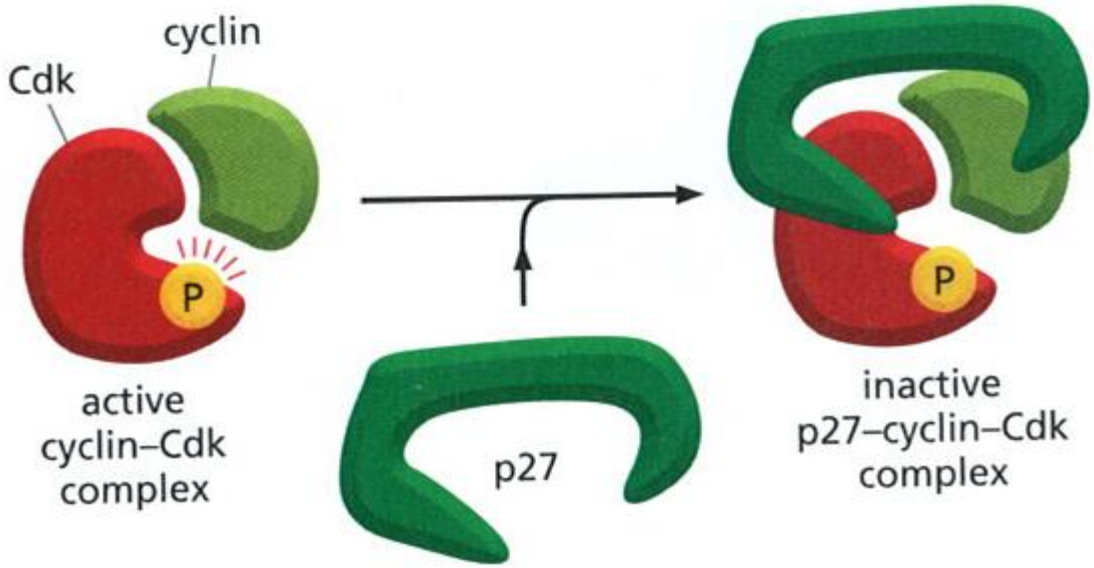
P-T-loop improves binding of substrate

Activation of Cdk kinase activity-Cdk2-Cyclin A



Inhibition of Cdk kinase activity:

-Binding of Cdk inhibitor proteins to Cyc/Cdk complexes

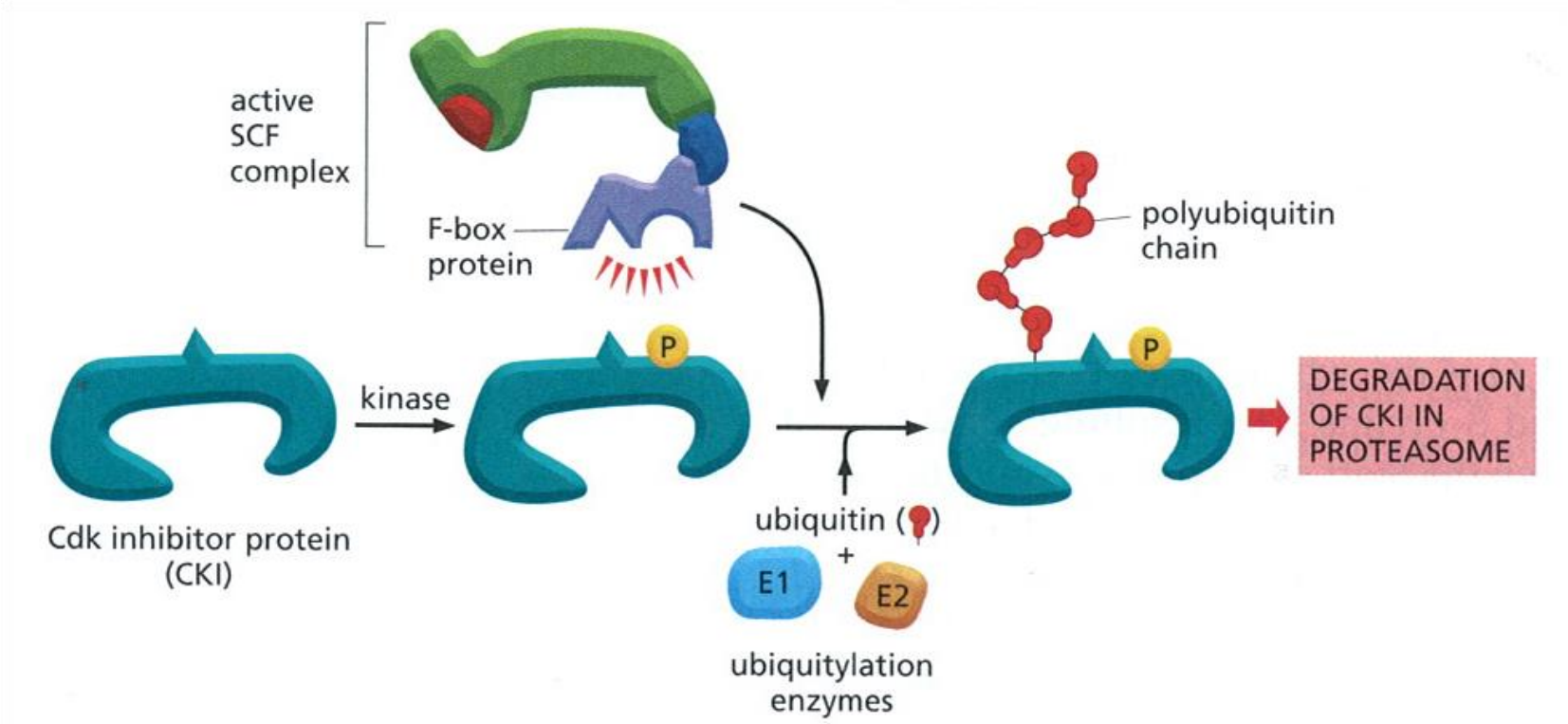


P27 binding distorts and binds into the active site of Cdk2
(for example inhibits G1/S-Cdk in G1 phase)

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:

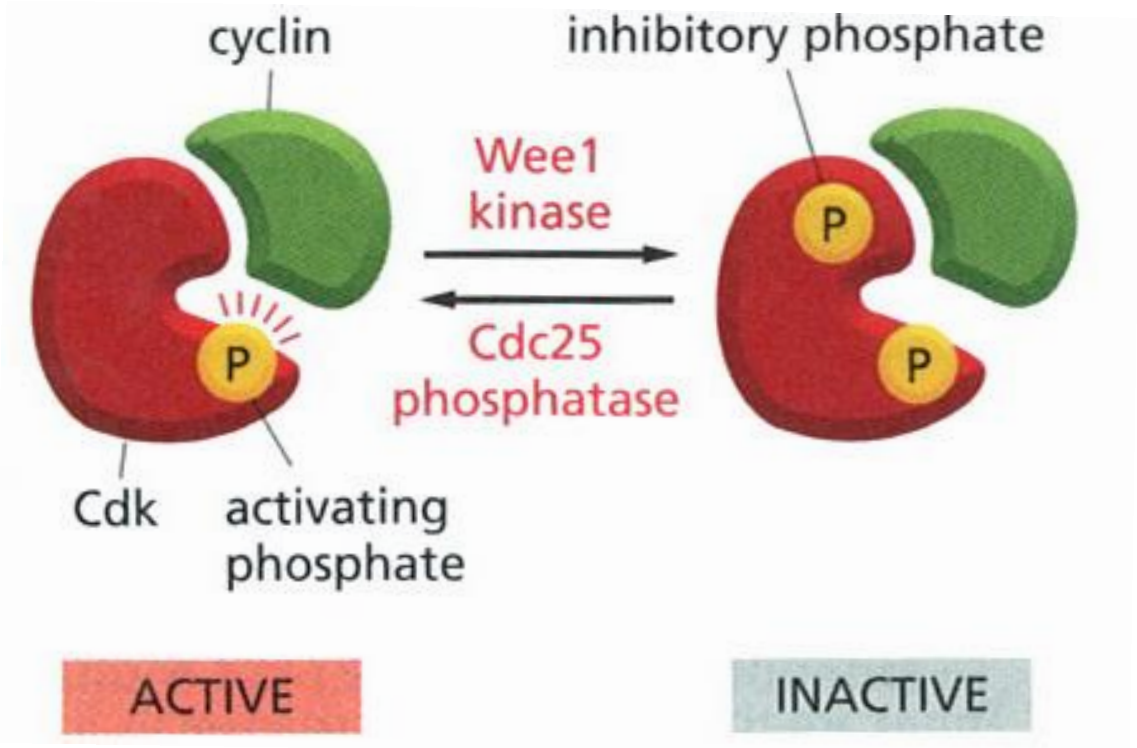
-Degradation of Cdk inhibitor proteins by ubiqitination and proteolysis



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

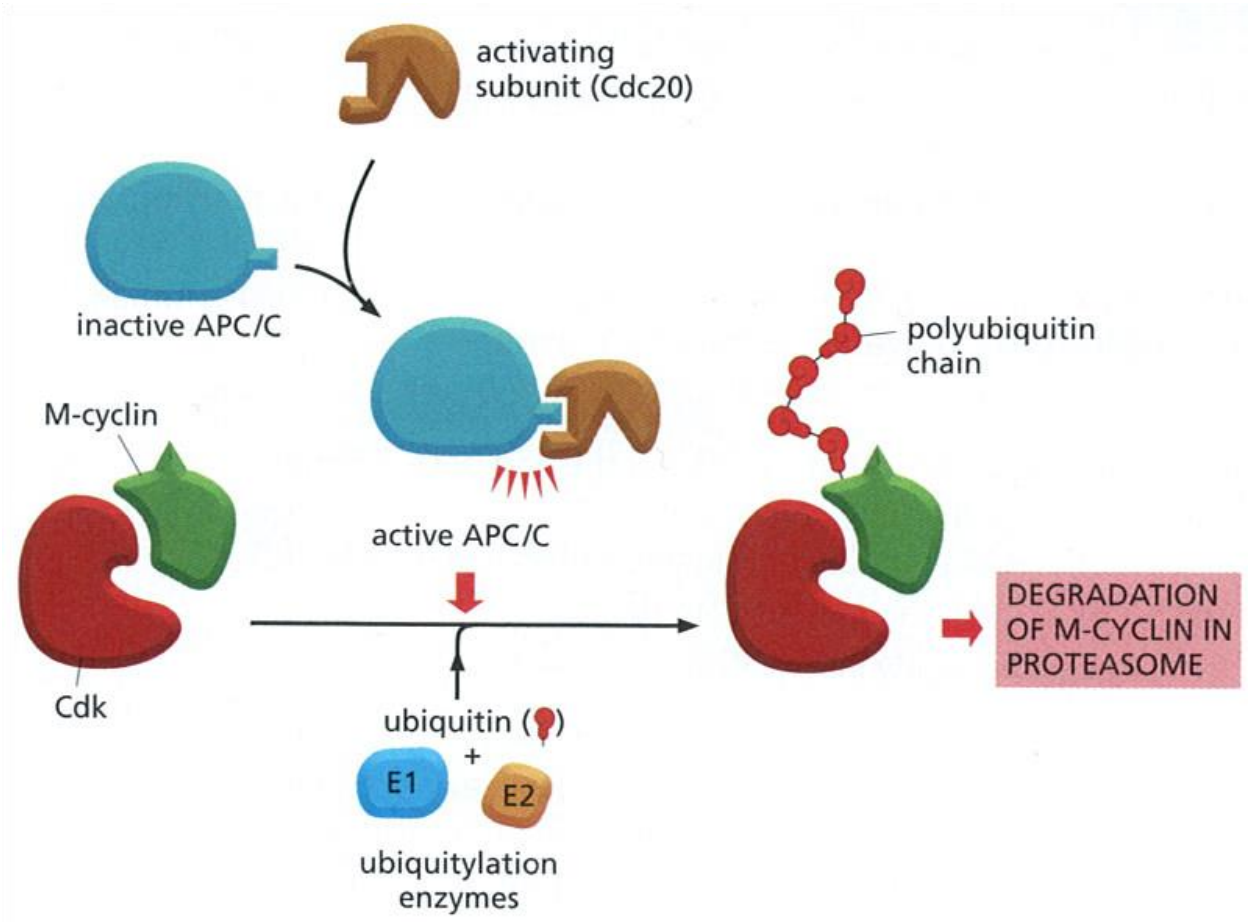
Inhibition of Cdk kinase activity:

-Inhibitory phosphorylation of Cdk



Inhibition of Cdk kinase activity:

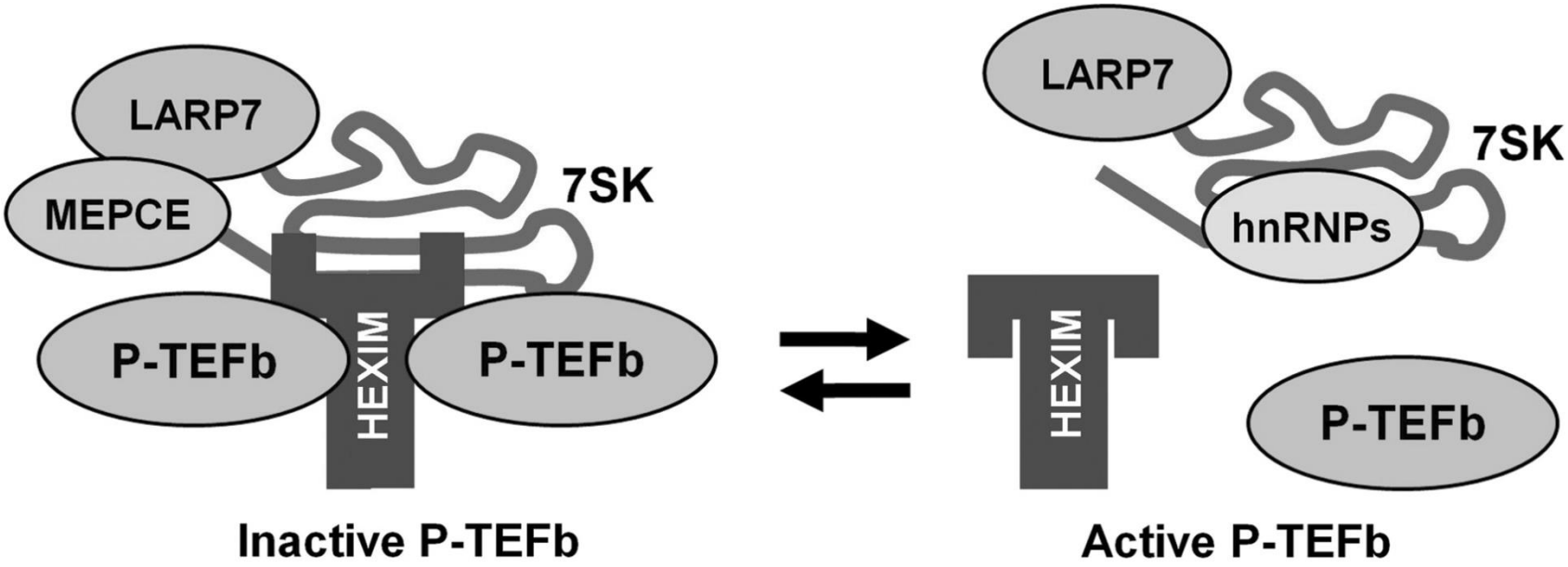
-Ubiquitination and degradation of Cyclin by proteasome



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

Inhibition of Cdk kinase activity:

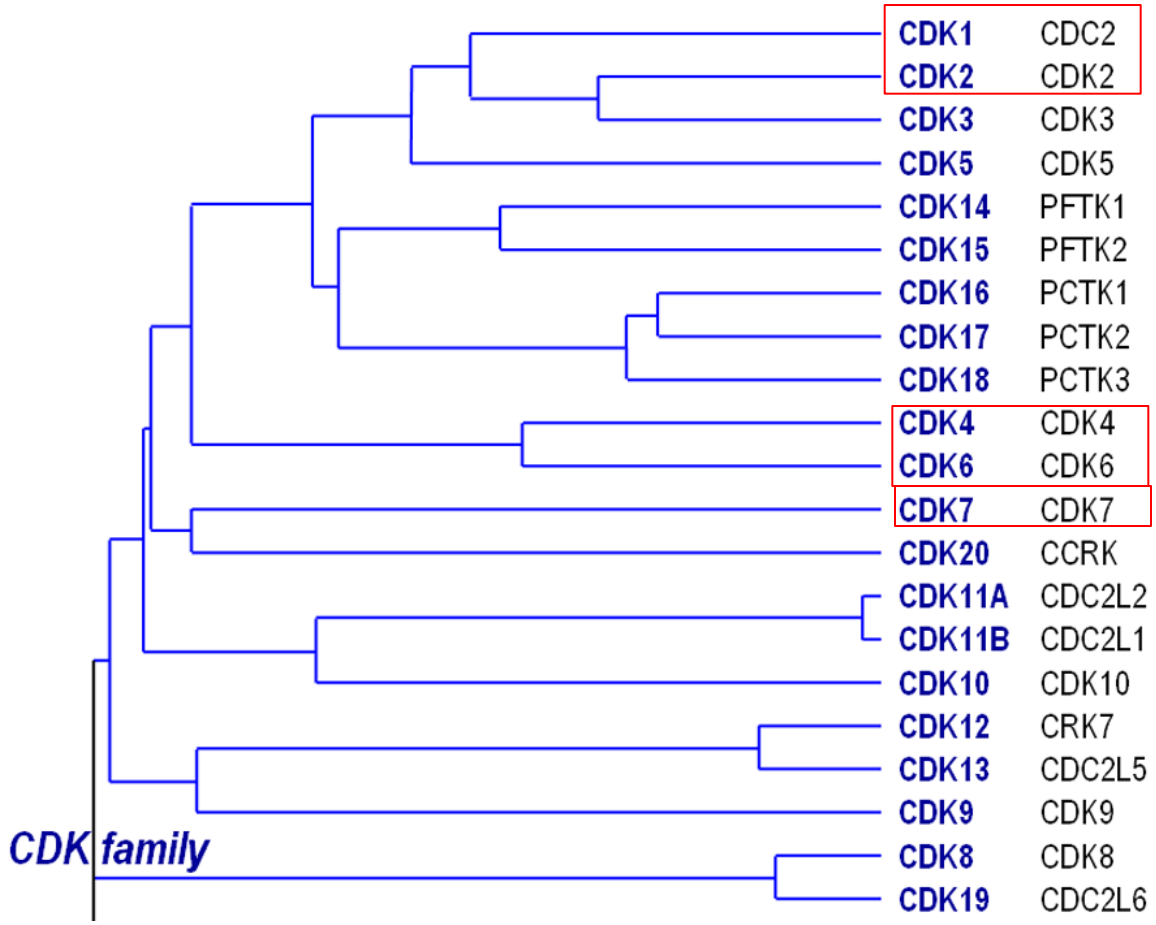
-Binding of Cdk inhibitor proteins and 7SK small nuclear RNA (7SK snRNA) to CycT/Cdk9 complex



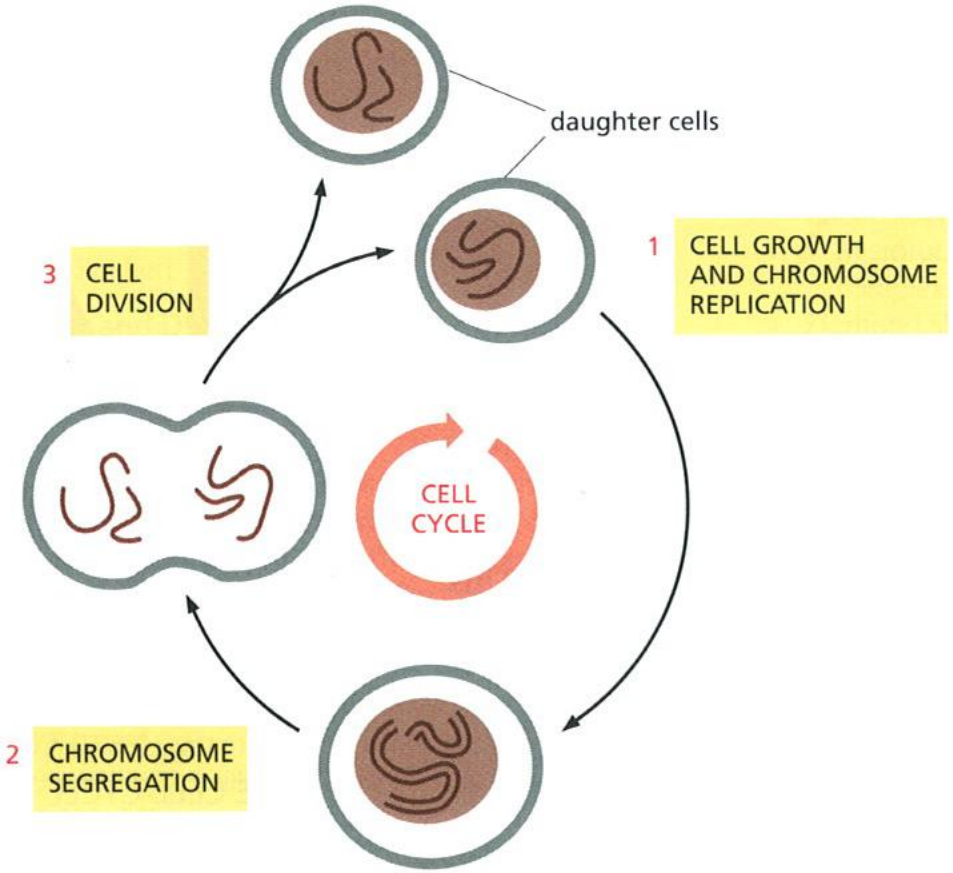
P-TEFb=Cdk9

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

Regulation of Cell Cycle by Cdks

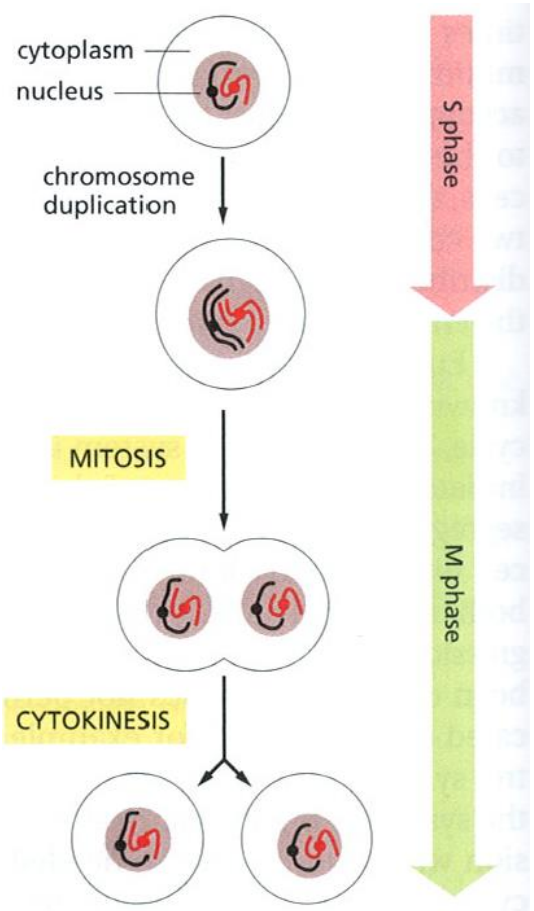


Cell Cycle



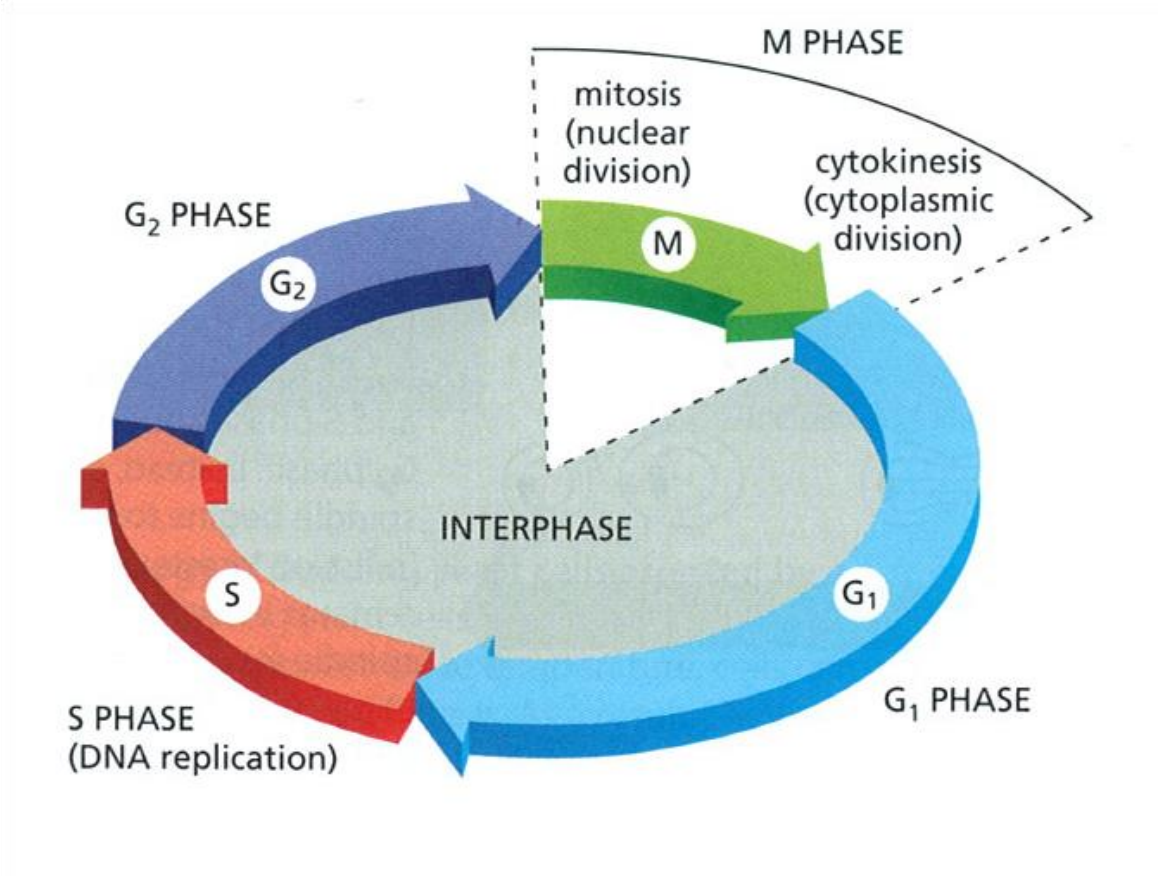
Cell cycle leads to production of two genetically identical daughter cells

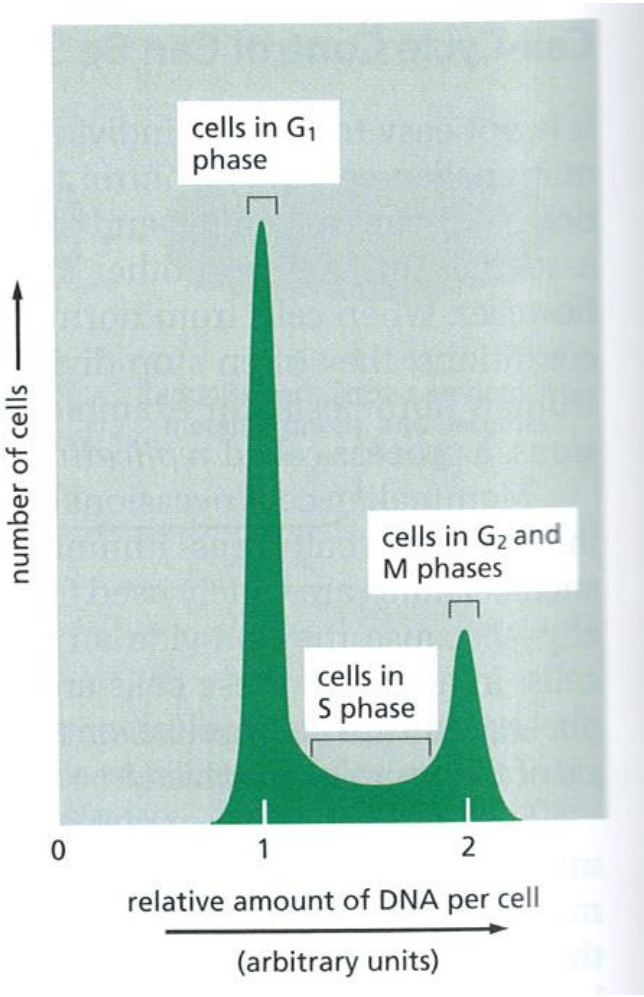
Major events of the cell cycle



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

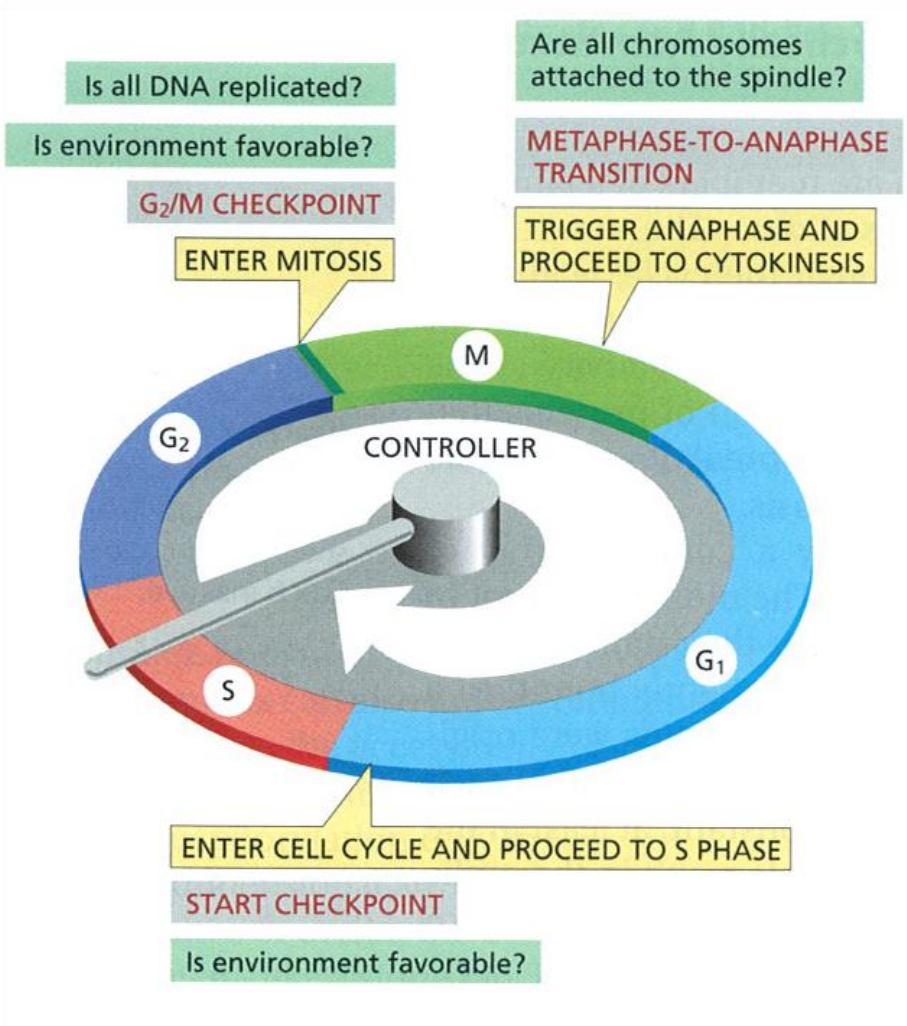




G1 and G2 phases-time delay to allow the growth of the cell

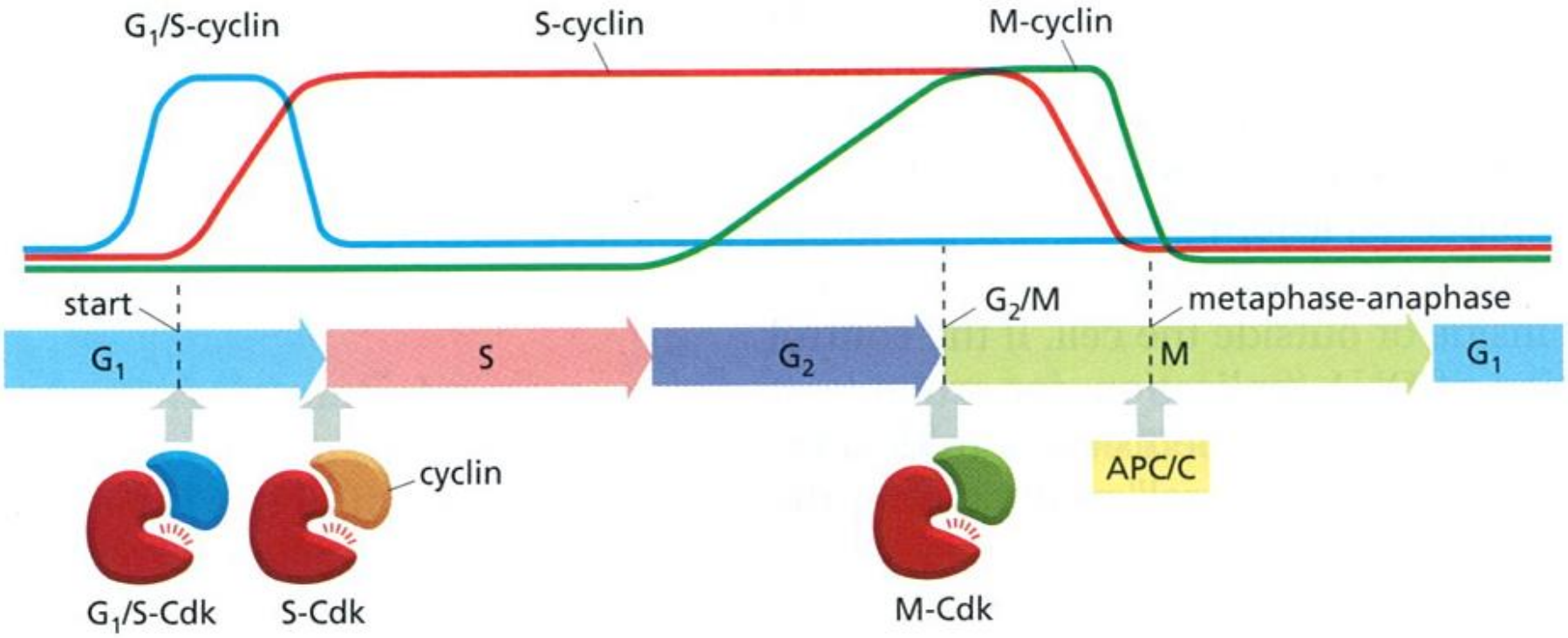
-time to monitor external and internal conditions before commitment to onset of S and M phase

The control of the cell cycle-three major checkpoints



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

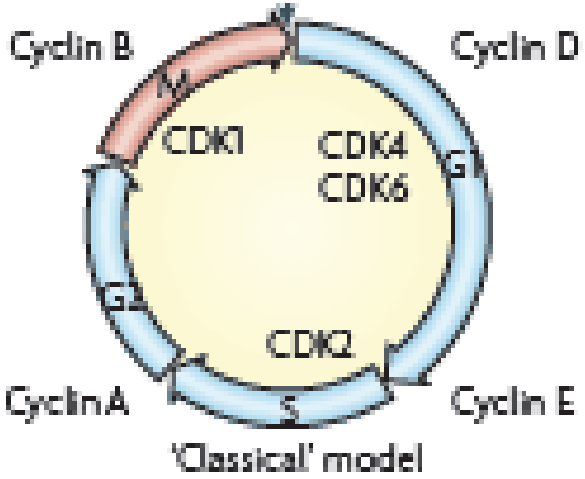
Cell cycle control system depends on cyclically activated Cdks



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



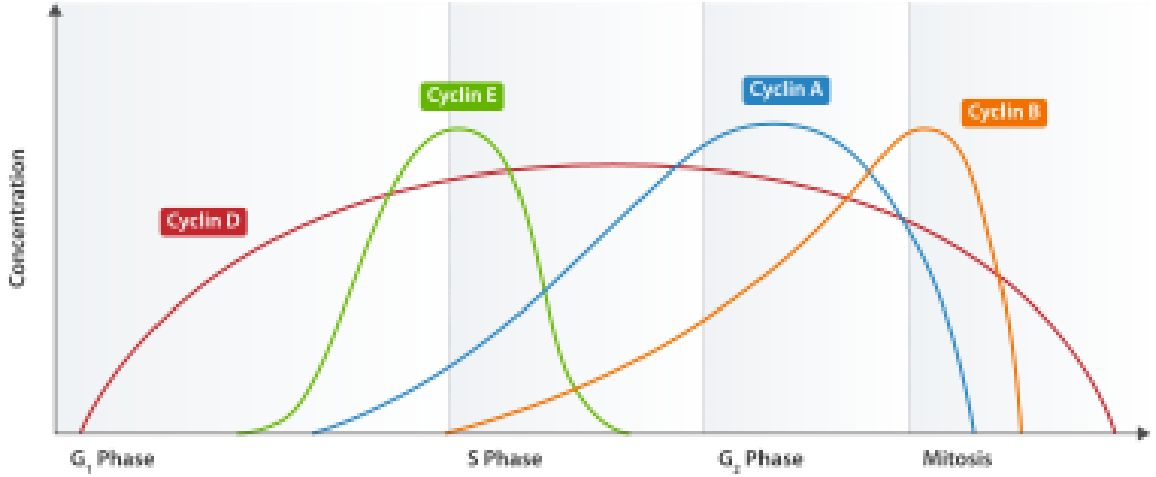
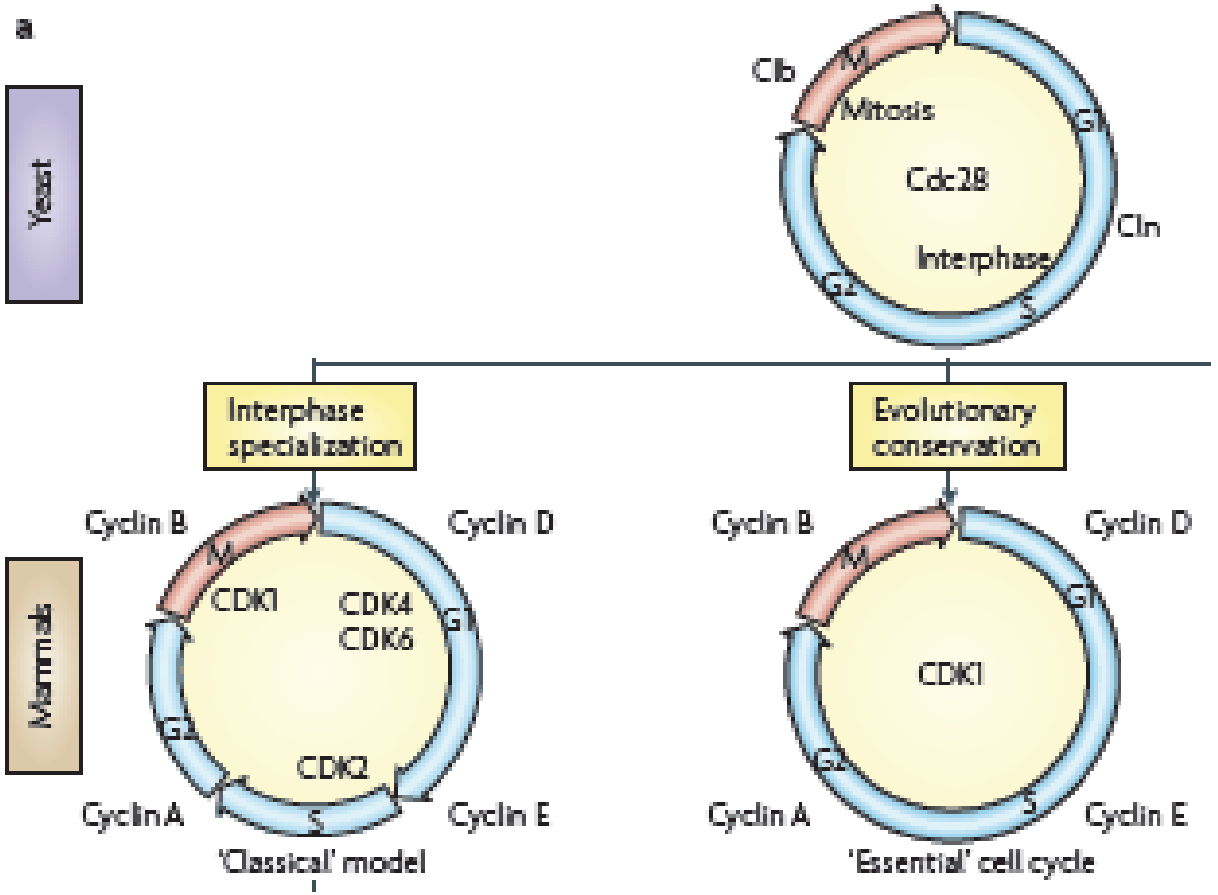


Table 17–1 The Major Cyclins and Cdks of Vertebrates and Budding Yeast

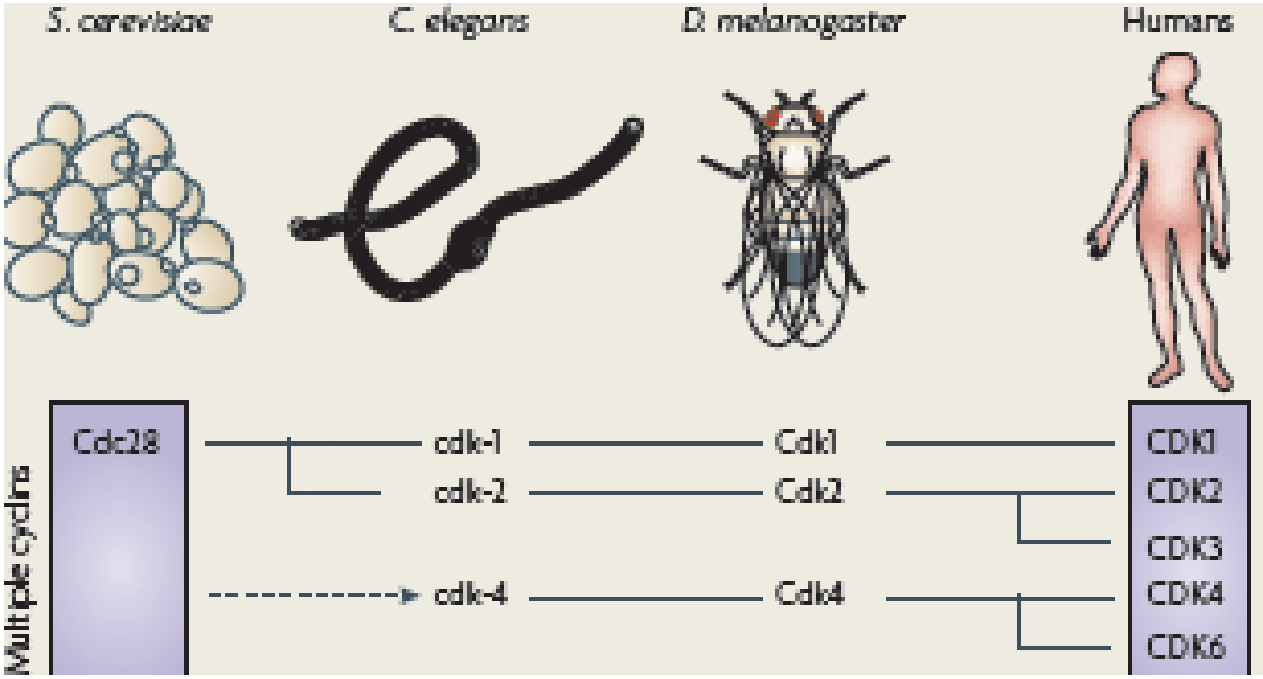
CYCLIN–CDK COMPLEX	VERTEBRATES		BUDDING YEAST	
	CYCLIN	CDK PARTNER	CYCLIN	CDK PARTNER
G_1-Cdk	cyclin D*	Cdk4, Cdk6	Cln3	Cdk1**
G_1/S-Cdk	cyclin E	Cdk2	Cln1, 2	Cdk1
S-Cdk	cyclin A	Cdk2, Cdk1**	Clb5, 6	Cdk1
M-Cdk	cyclin B	Cdk1	Clb1, 2, 3, 4	Cdk1

Comparison of the yeast and mammalian cell cycle

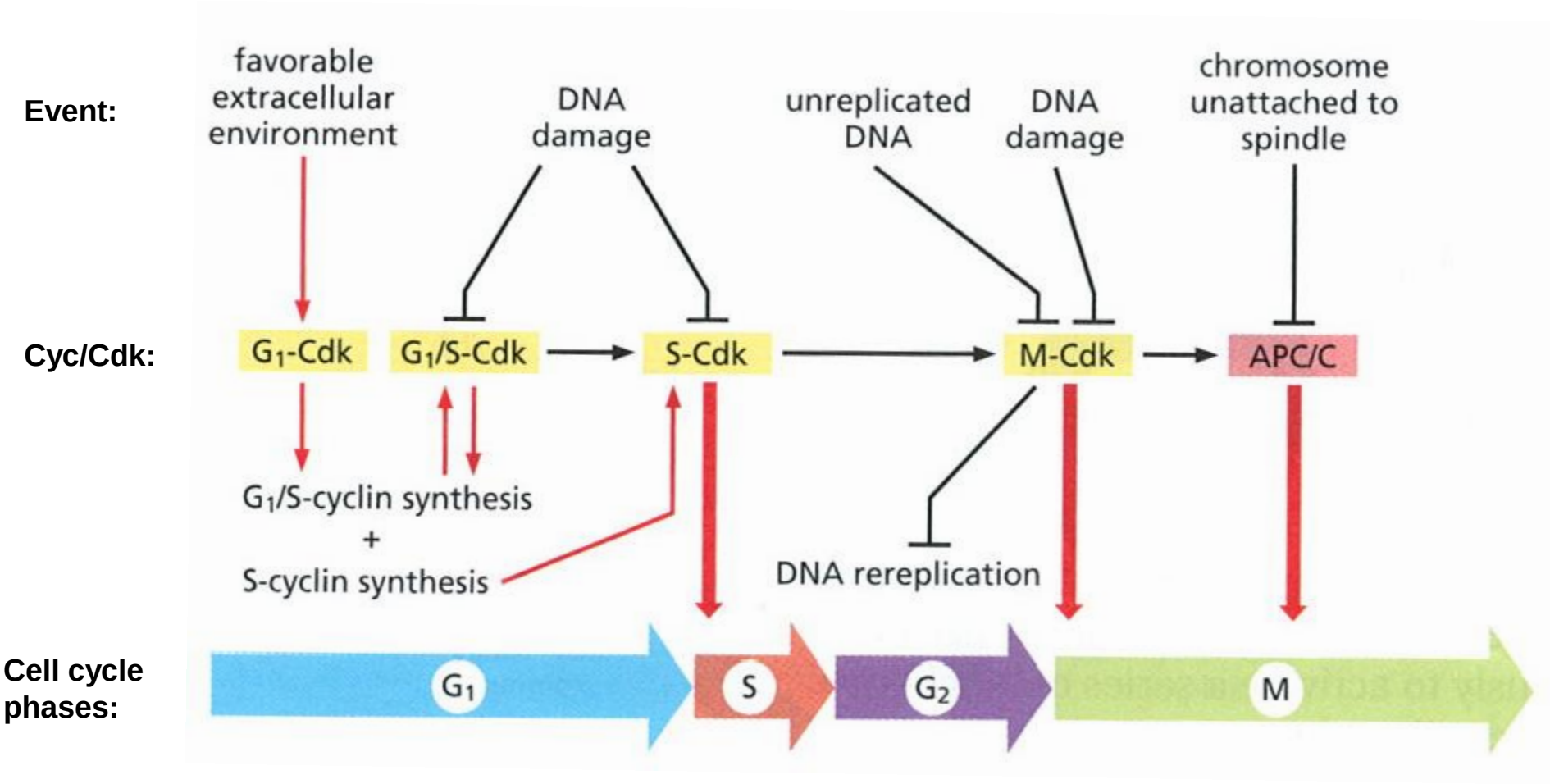


Yeast- cell cycle is directed by one Cdk-Cdk1 (cdc28)
Mammals-several Cdks (classical model), Cdk1 is essential to drive cell cycle in the absence of other Cdk (mouse knock out model)

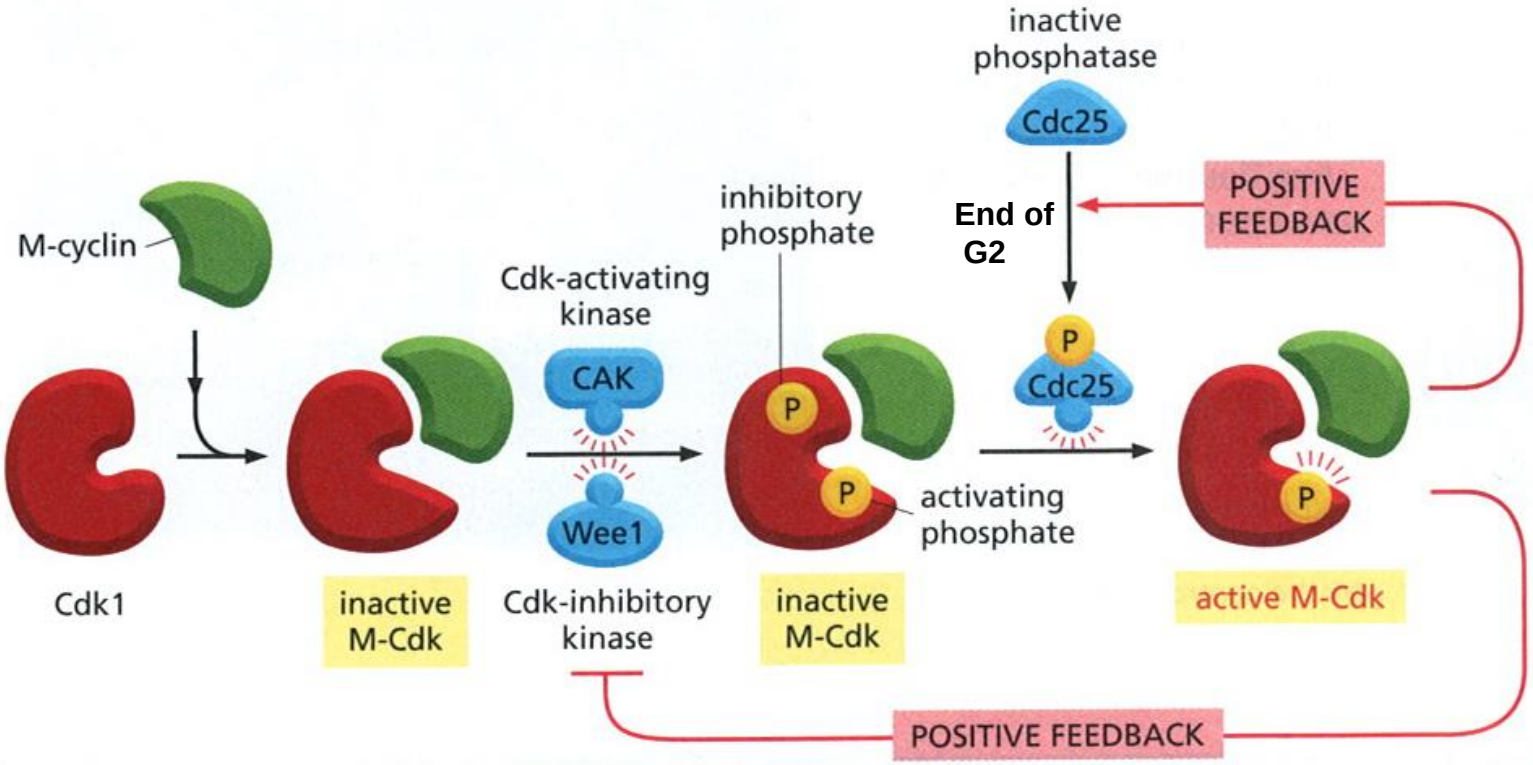
Evolution of cell cycle control

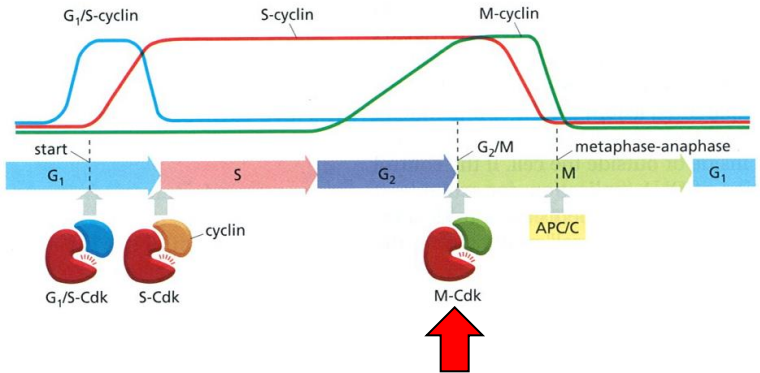


Cell cycle control system is a network of biochemical switches where Cyc/Cdk complexes play a major role



Activation of M-Cdk (cycB/cdk1)

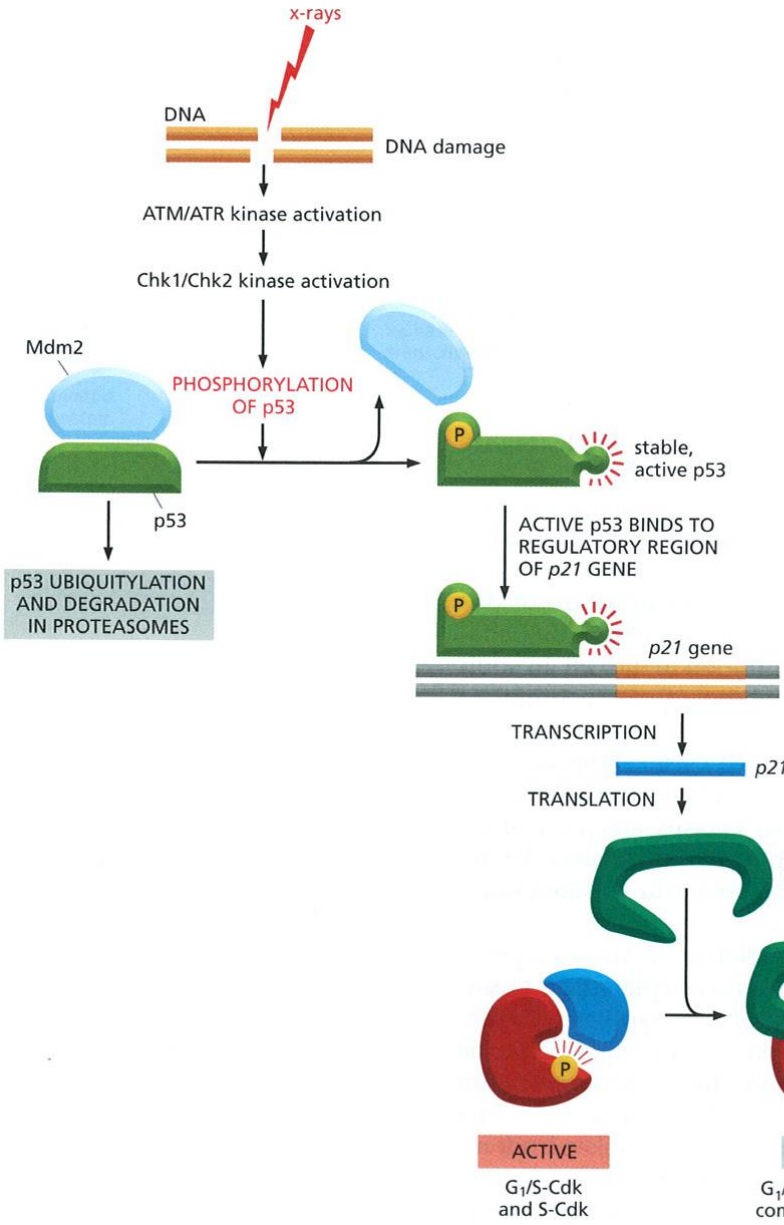


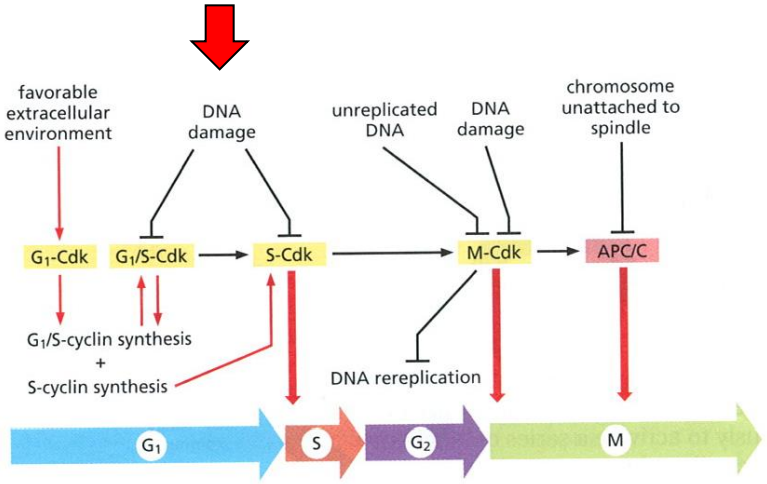


De-phosphorylation activates accumulated M-Cdk at the onset of mitosis

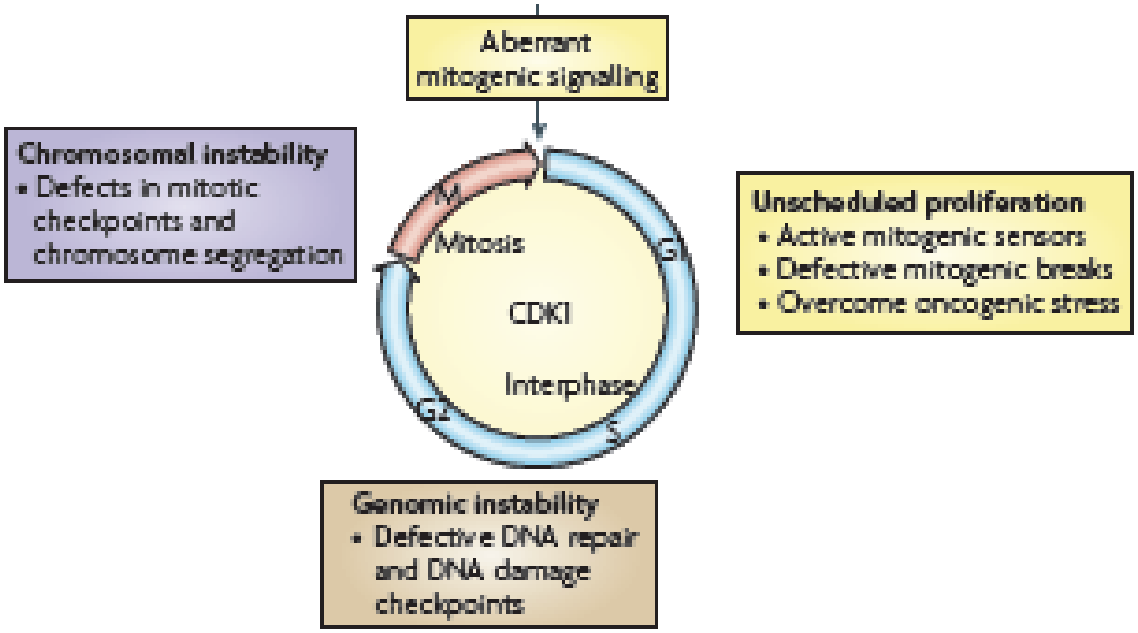
Mechanism of cell cycle arrest in G1 by DNA damage

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



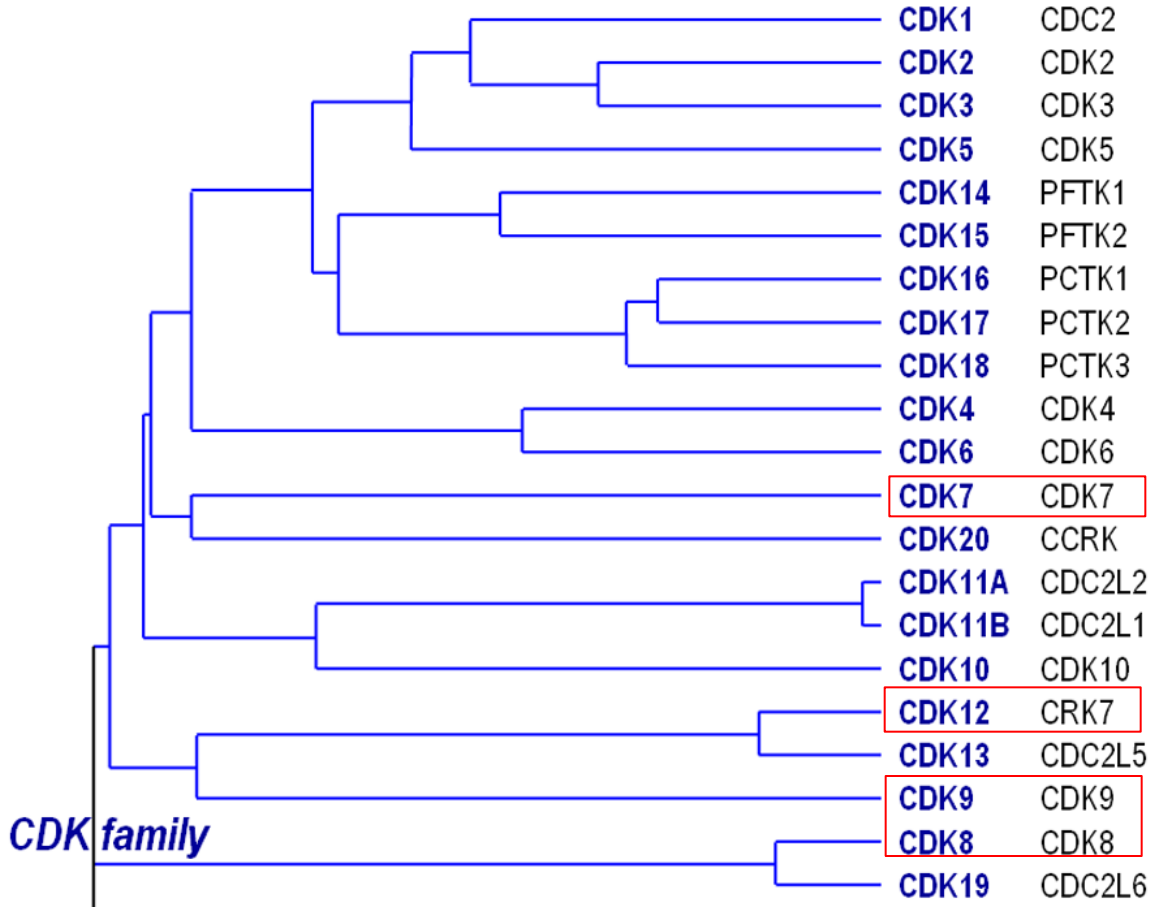


Deregulation of cell cycle and cancer

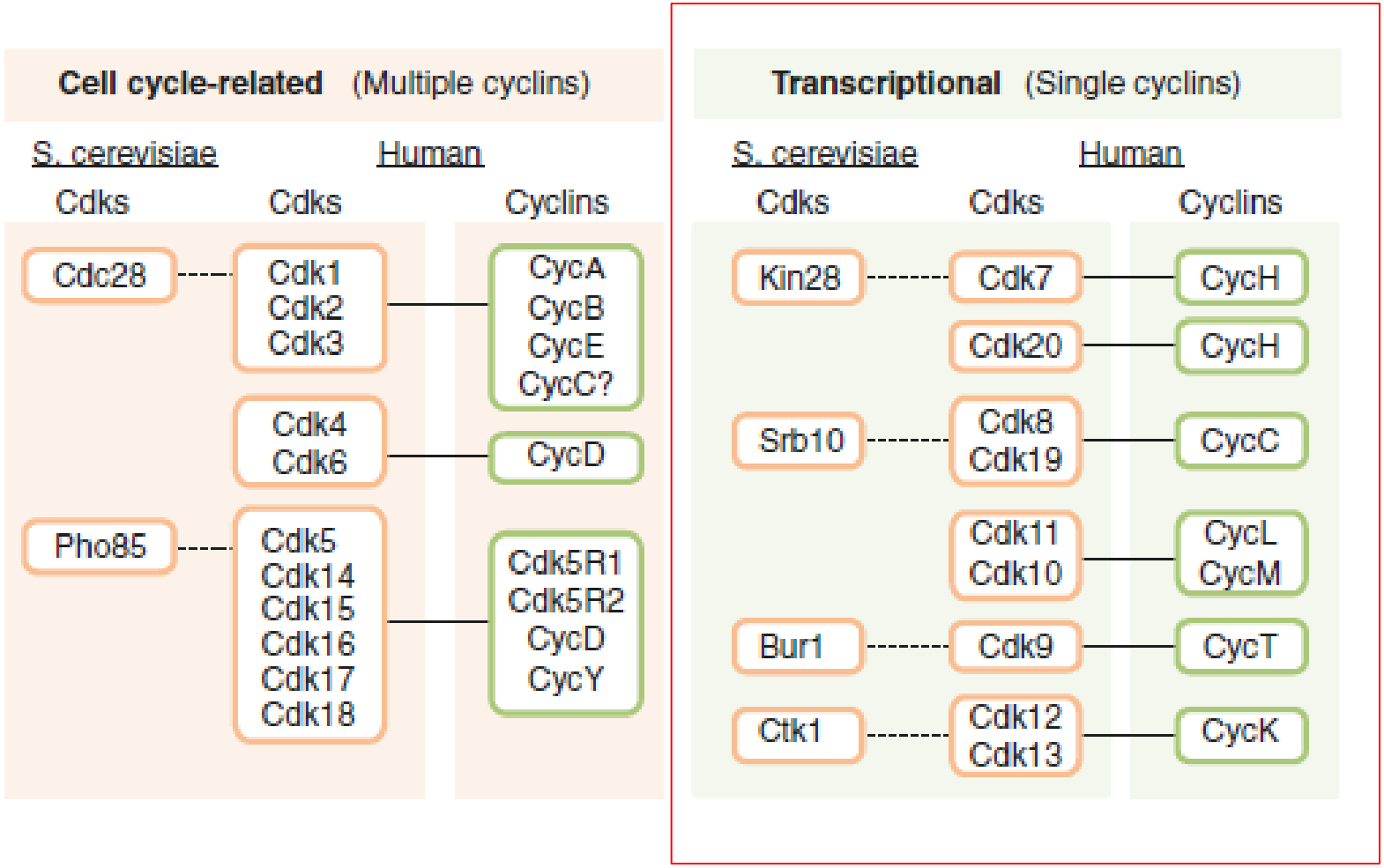


Cells escape from the proper control of the cell cycle during cancer development:
-Increase in expression and activity of proteins driving cell cycle regulators (Cdks)
-Inactivation of inhibitors of Cdks

Regulation of transcription by Cdks



Transcriptional Cyc/Cdk complexes



Major differences between Transcription and Cell Cycle Cyc/Cdk complexes

Trancription Cyc/Cdks complexes:

1)Cdk has usually only one Cyclin partner

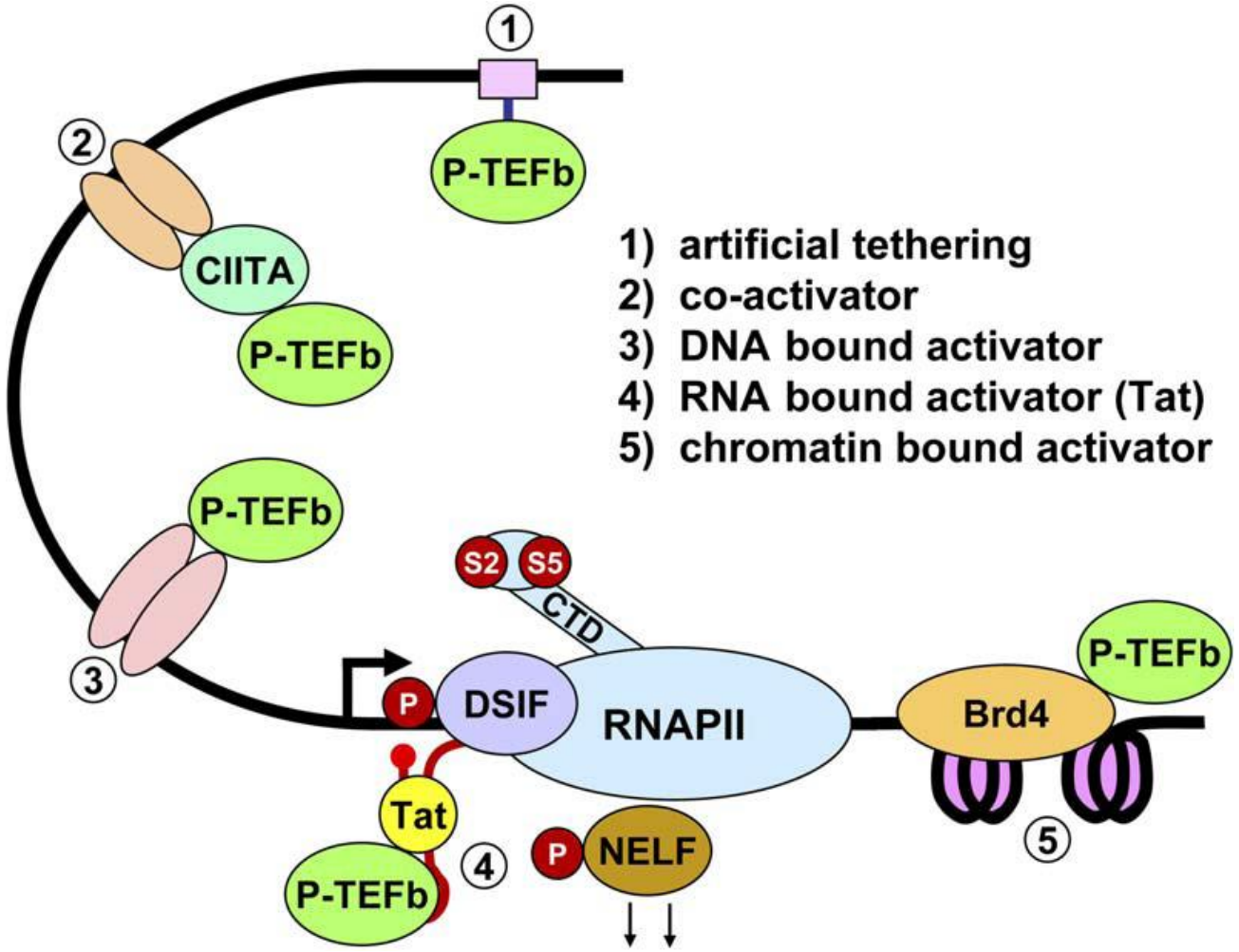
2)Usually in multi-protein complexes

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

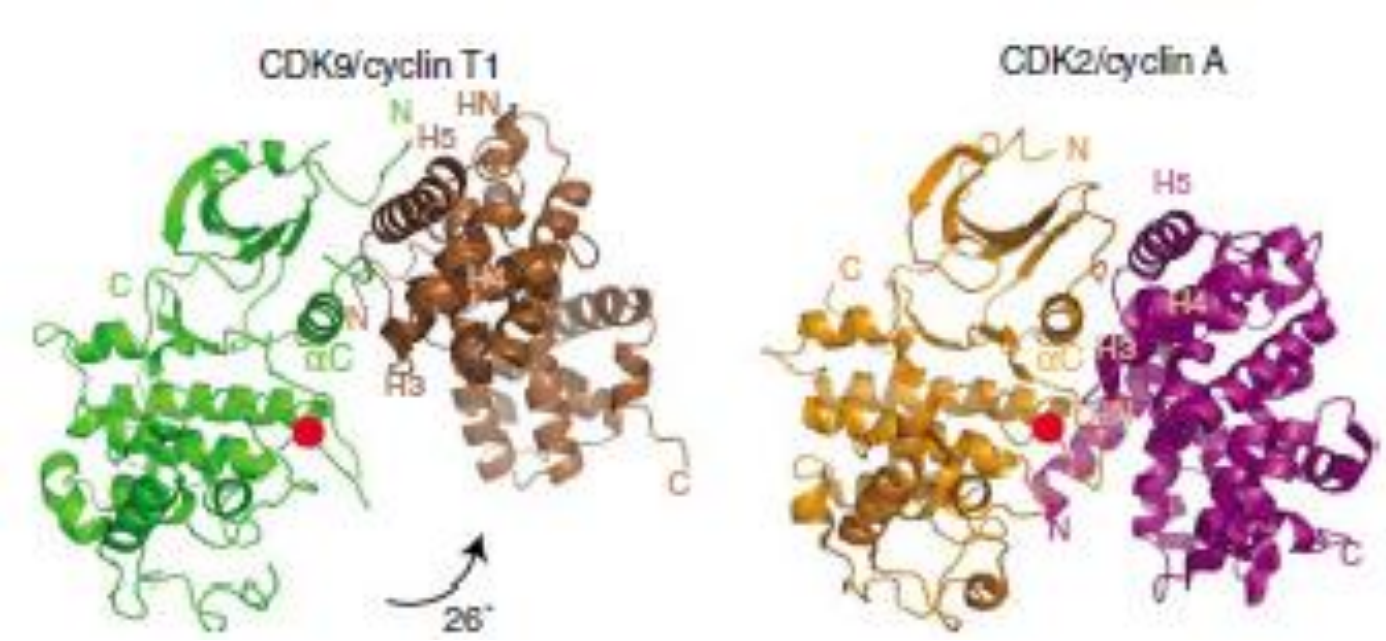
4)Regulated at the level of recruitment to specific gene

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

P-TEFb=Cdk9

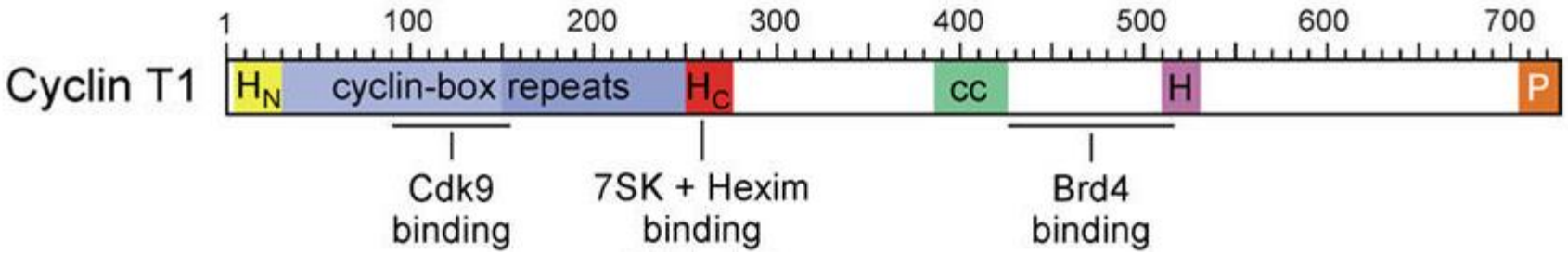


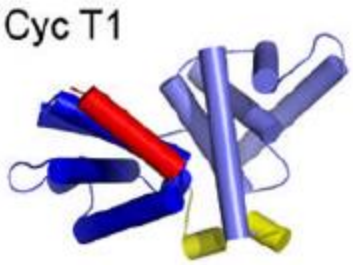
Differences between Cell Cycle and Transcription Cyc/Cdks-structure

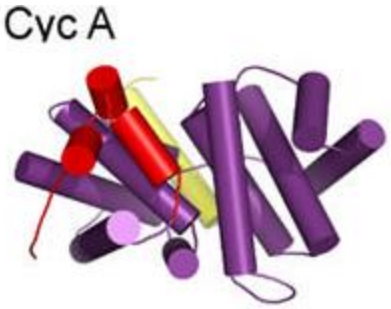


Sparse number of contacts btw Cyc and Cdk in transcription Cyc/Cdk complexes
More contacts in Cell Cycle Cyc/Cdk complexes - important for Cdk activation

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







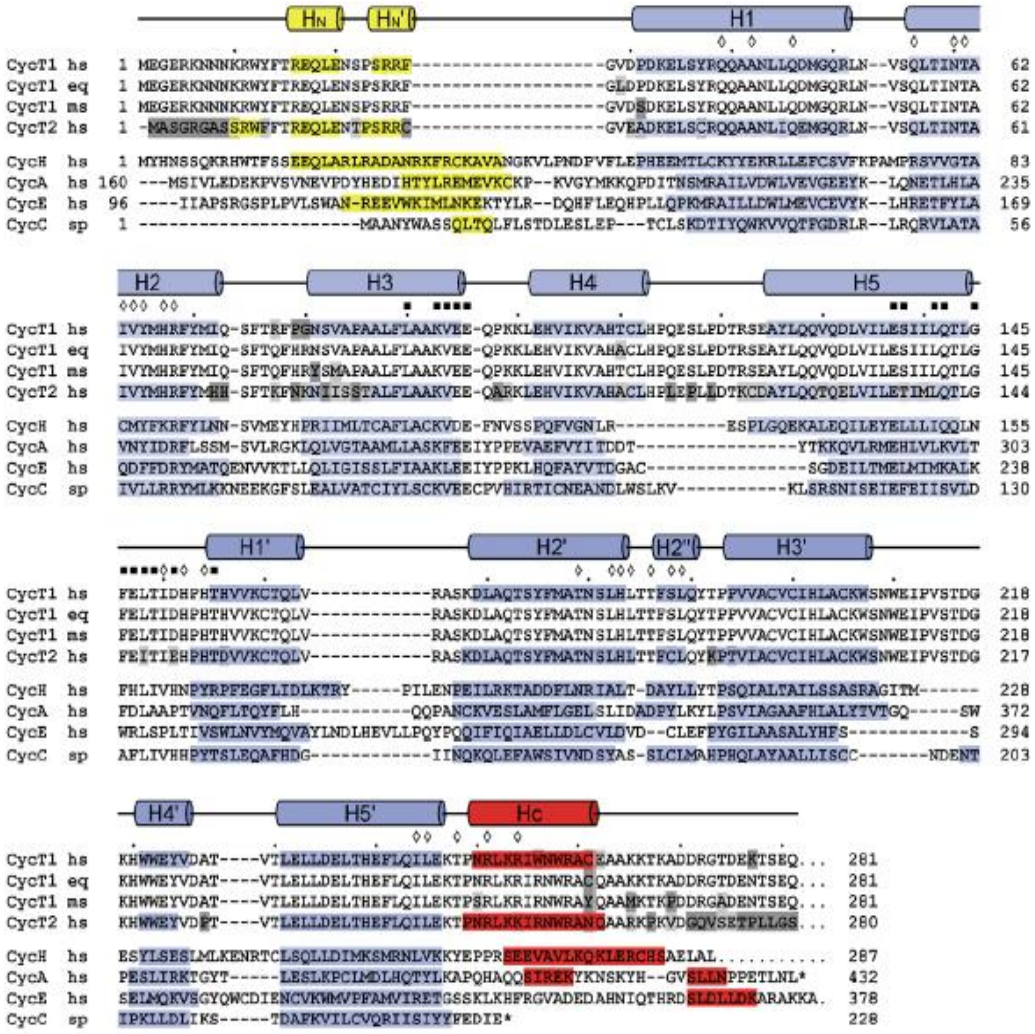
All Cyclins have <u>2 canonical cyclin-boxes responsible for Cdk binding</u>

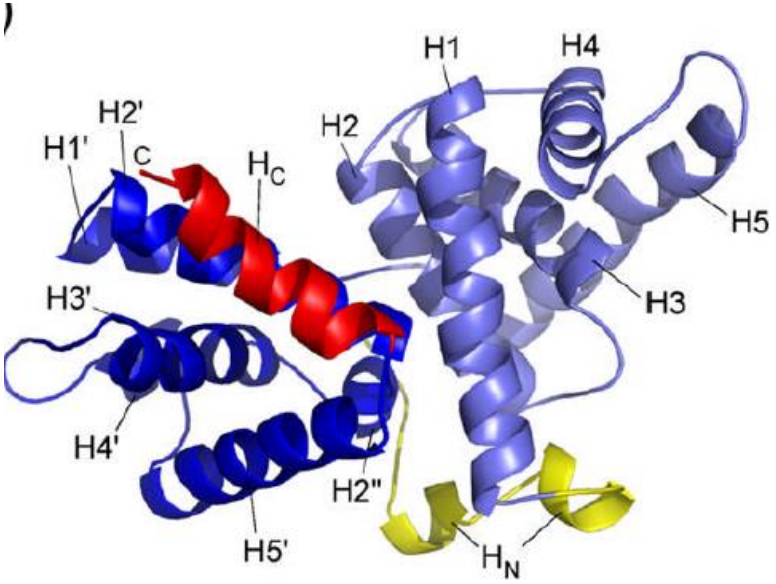
Each cyclin-box consists of 5 helixes

The <u>cyclin-boxes conserved in all Cyclins</u>

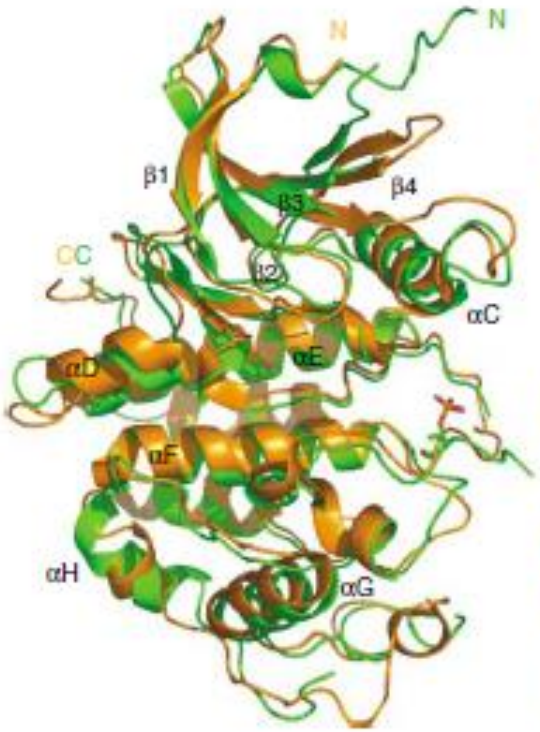
<u>Cell Cycle and Transcription Cyclins differ significantly in sequence and structure outside of the cyclin boxes (binding to other proteins)</u>

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

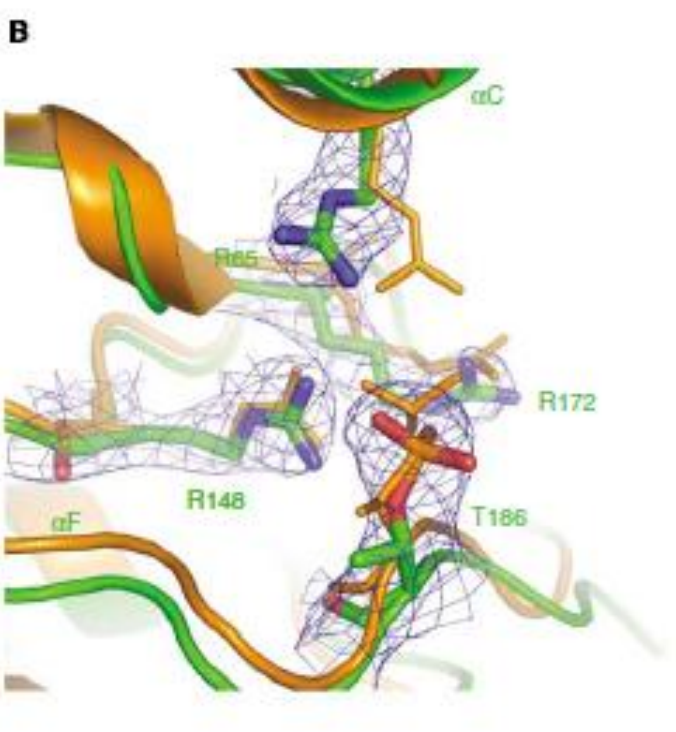




Comparison of Cdk9 and Cdk2



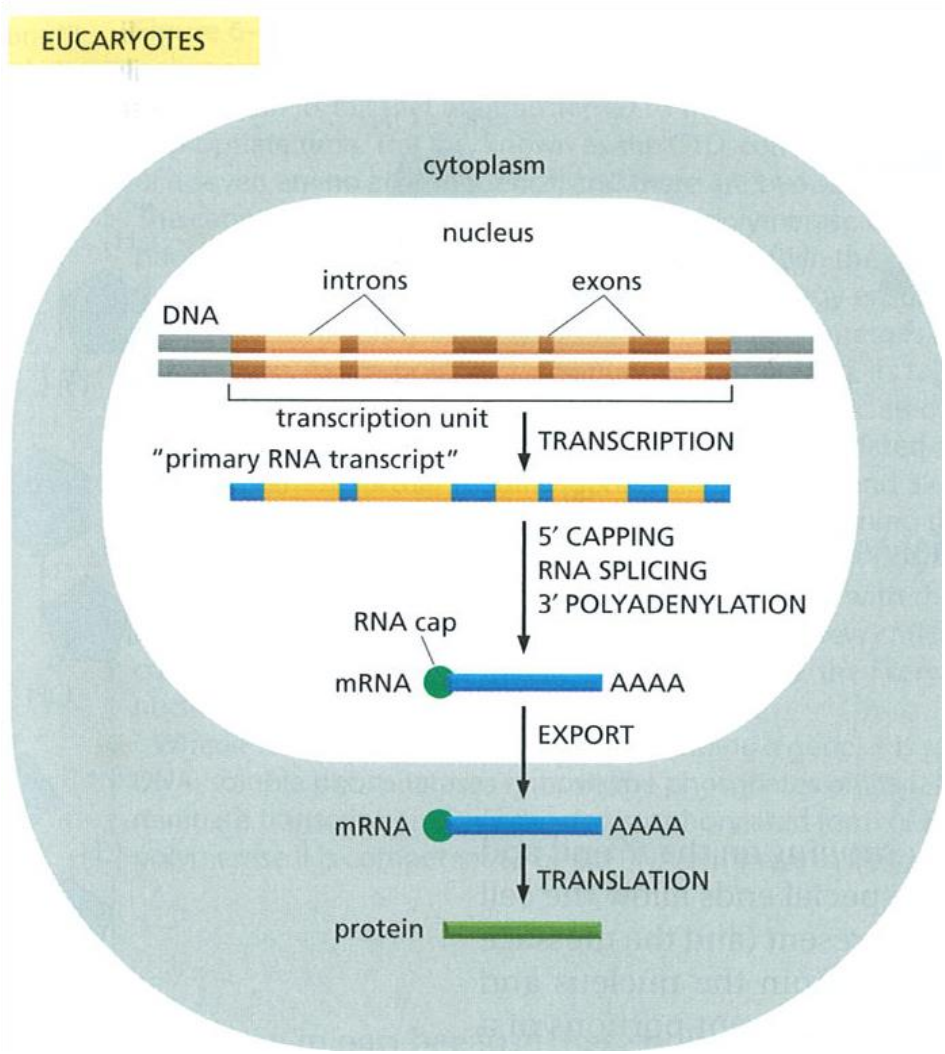
Cdk9 (green) /Cdk2 (orange)



T-loop (T186/T180)

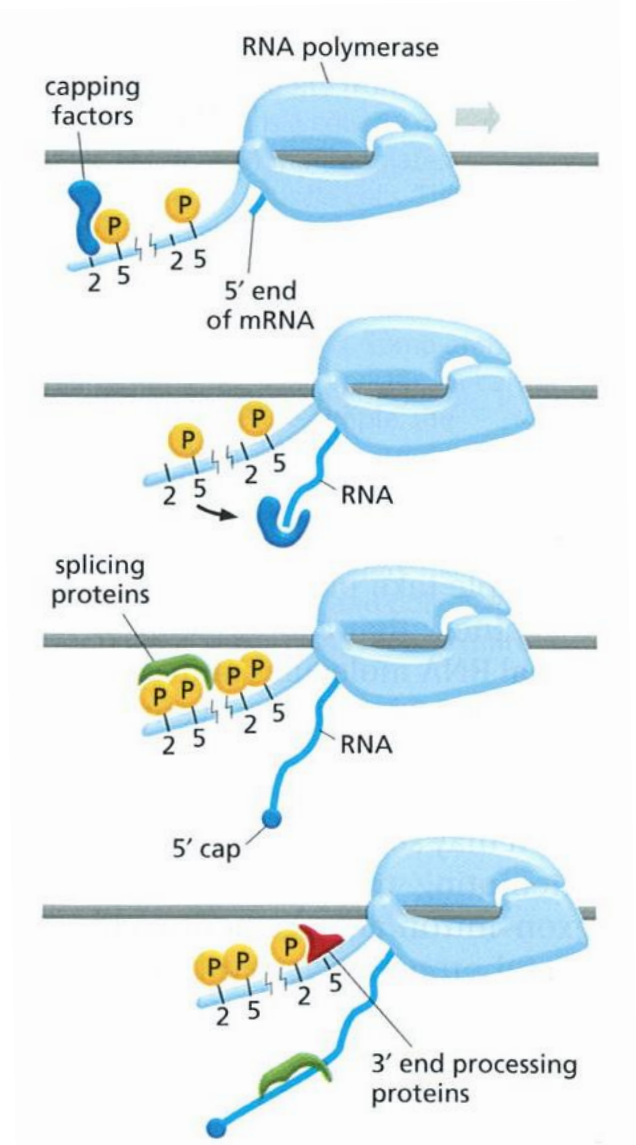
Structures very similar, sequence similarity 40%

Transcription (Gene expression)



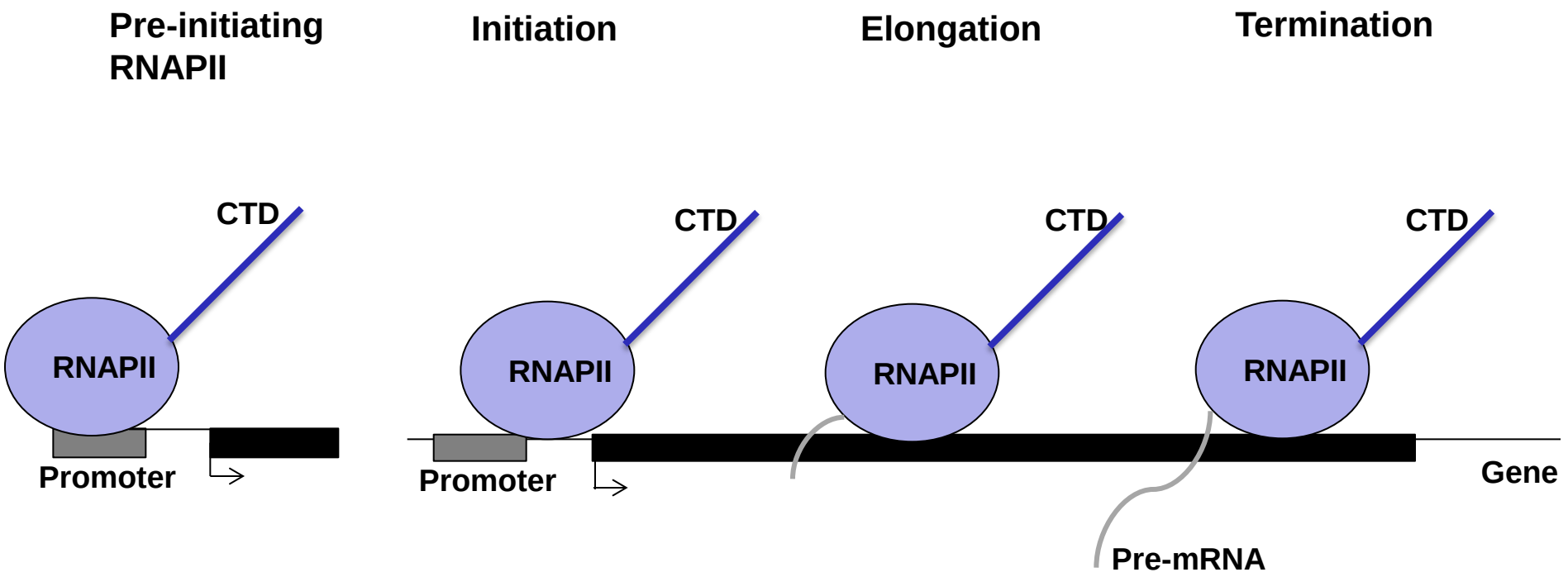
Transcription- synthesis of RNA from DNA template

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



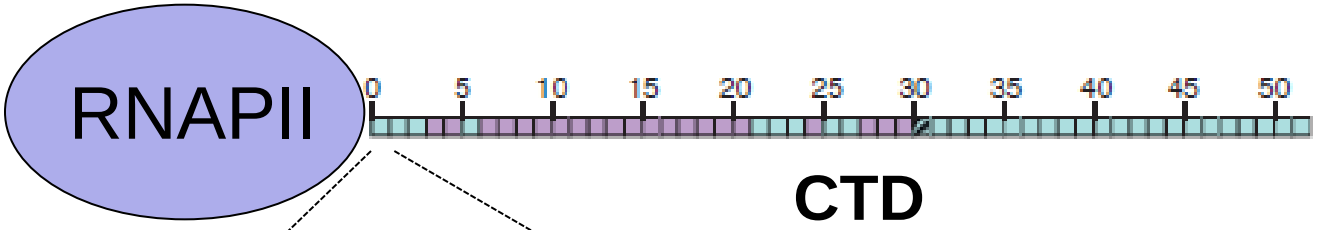
The co-transcriptional mRNA processing (capping, splicing, 3` prime end processing)

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"

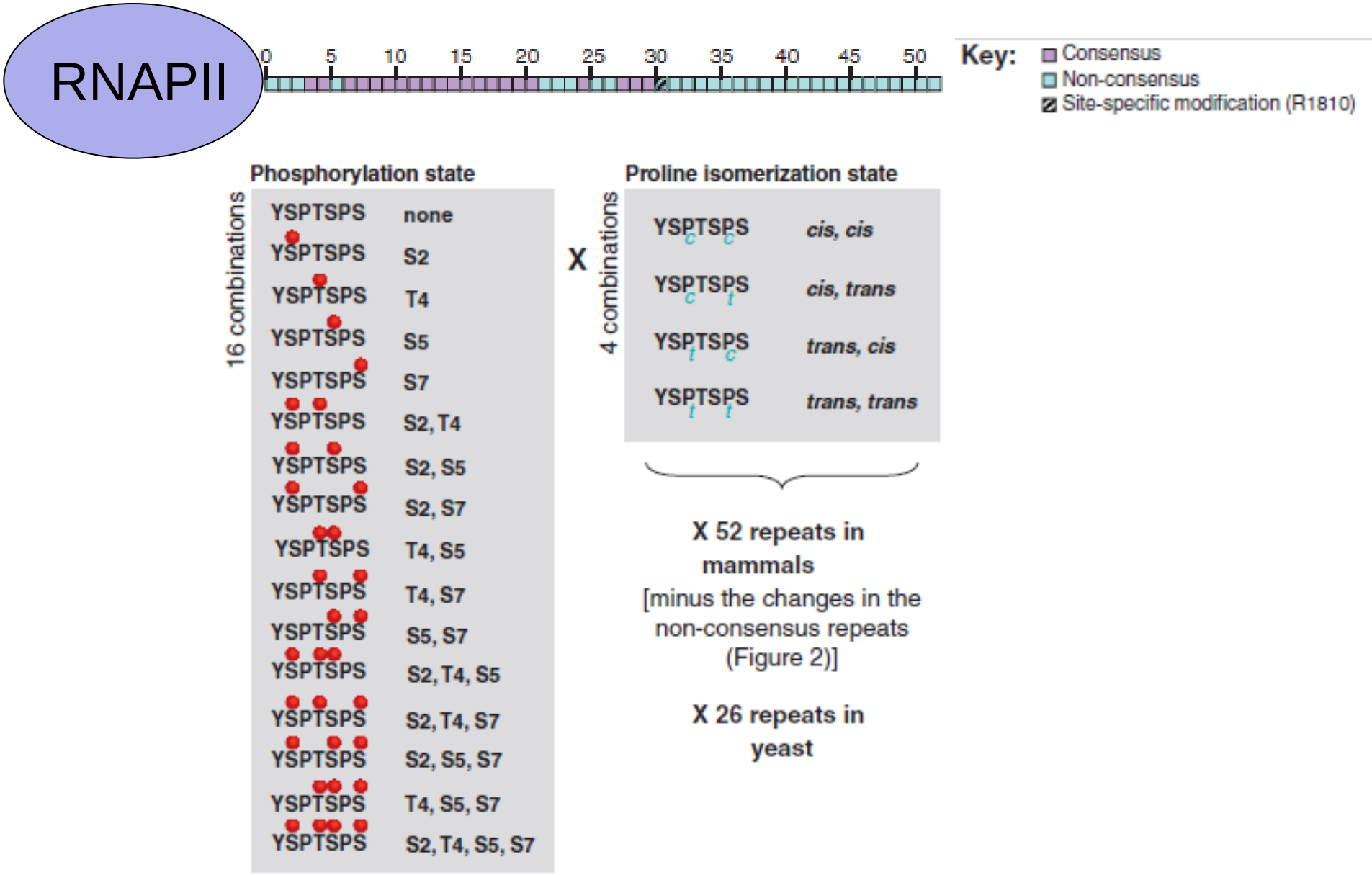


P P iso P P iso P

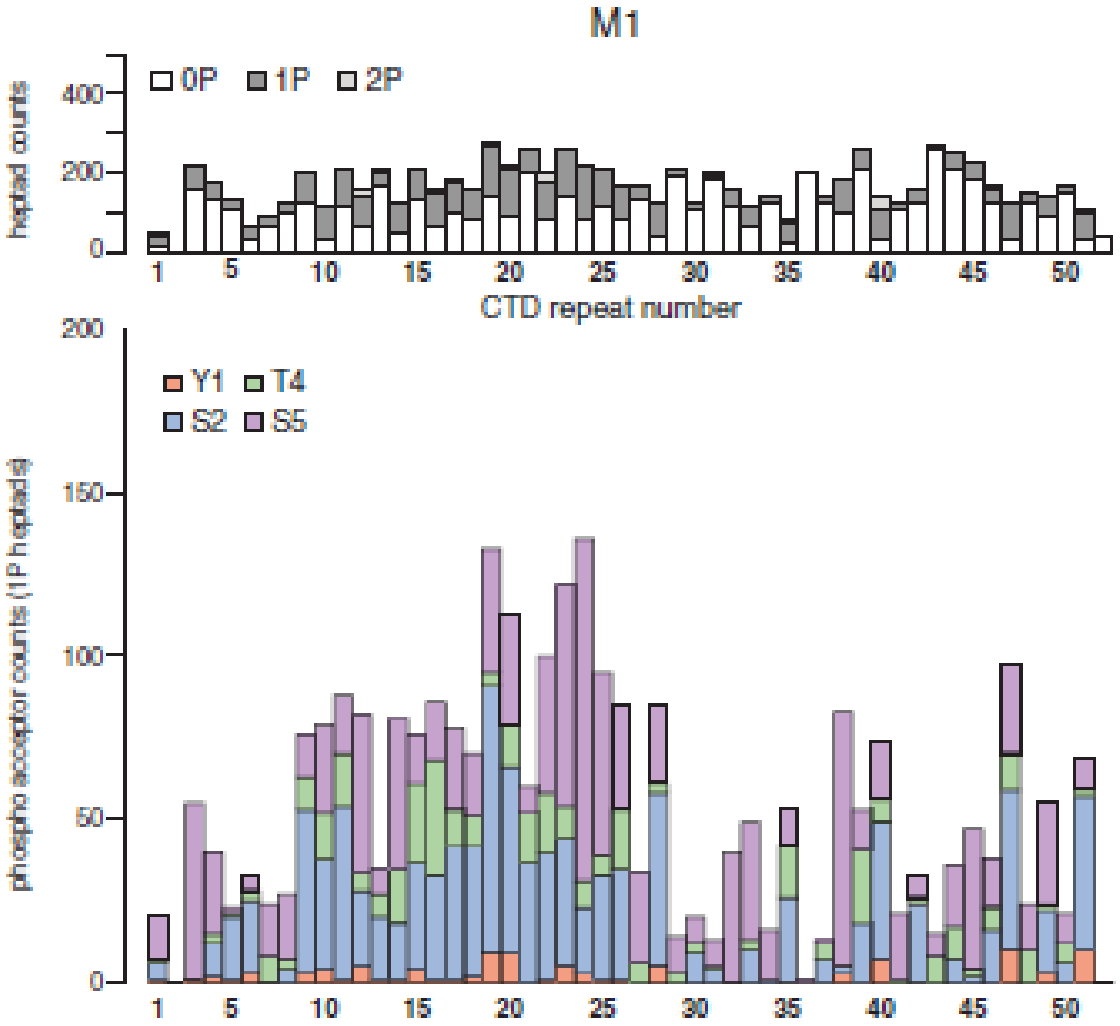
$(Y_1\text{-}S_2\text{-}P_3\text{-}T_4\text{-}S_5\text{-}P_6\text{-}S_7)x52$

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

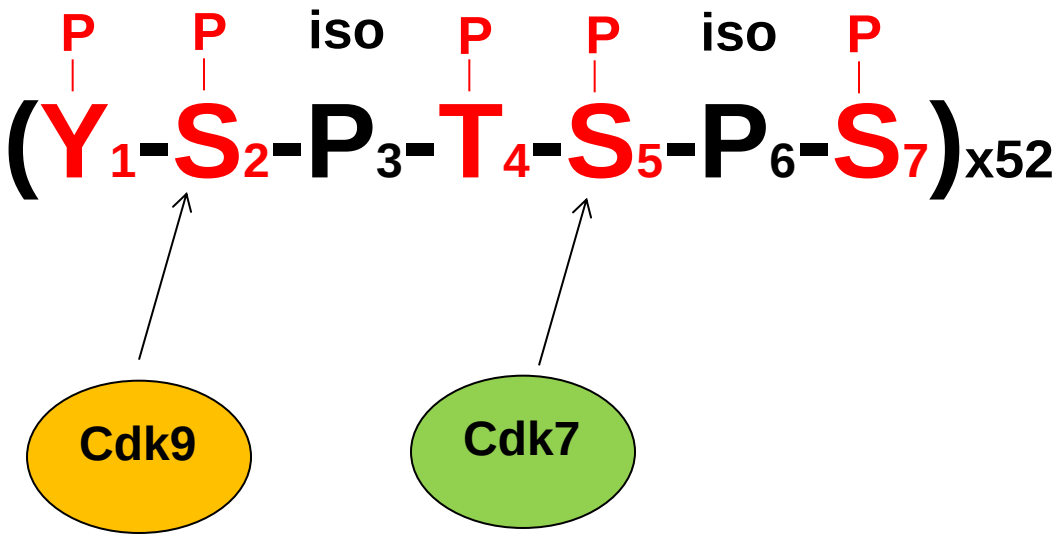
Human "CTD code"



Most of the CTD repeats carries 0-2 phosphorylations and serine5 and serine2 are most often phosphorylated

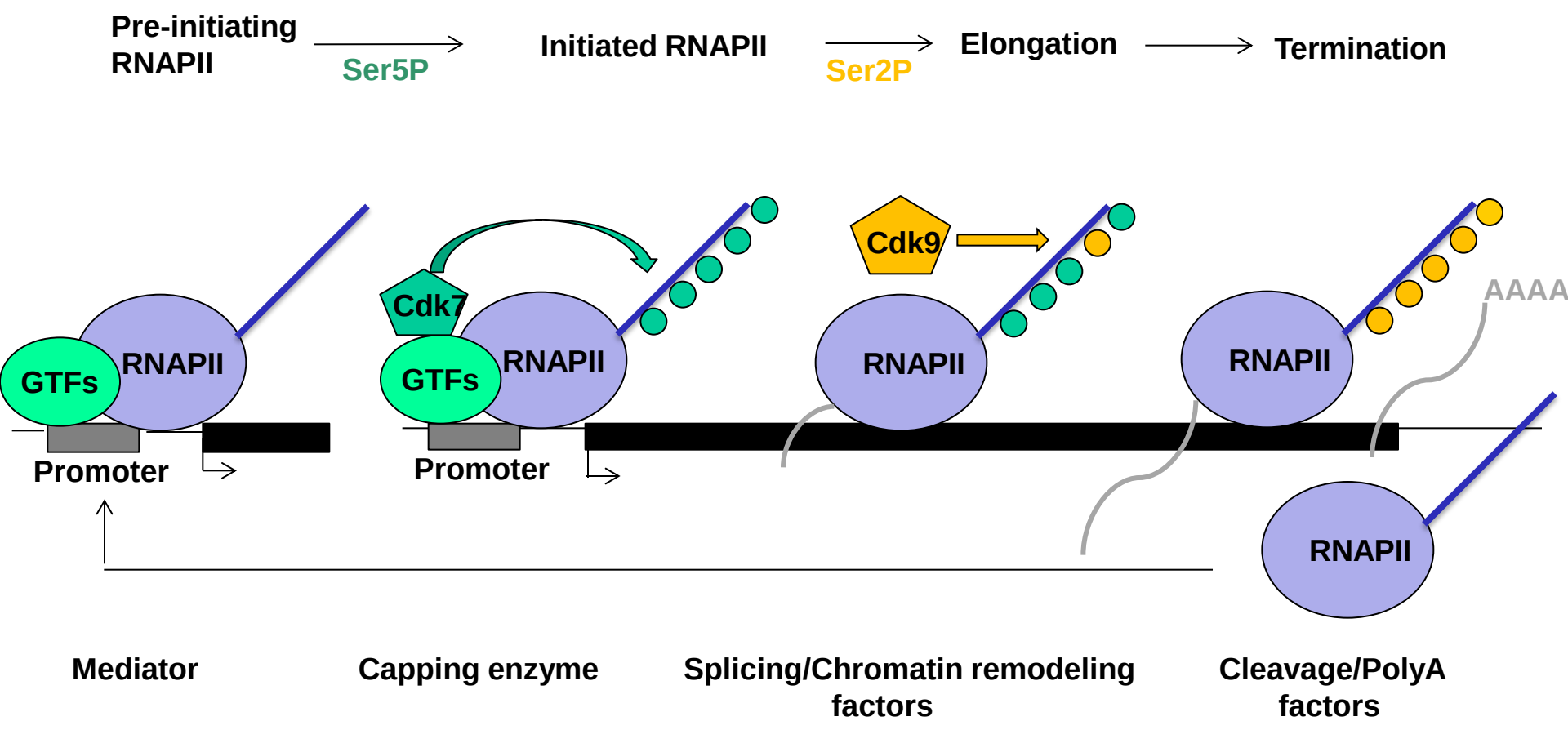


Repeats of the CTD get phosphorylated by the Cdks

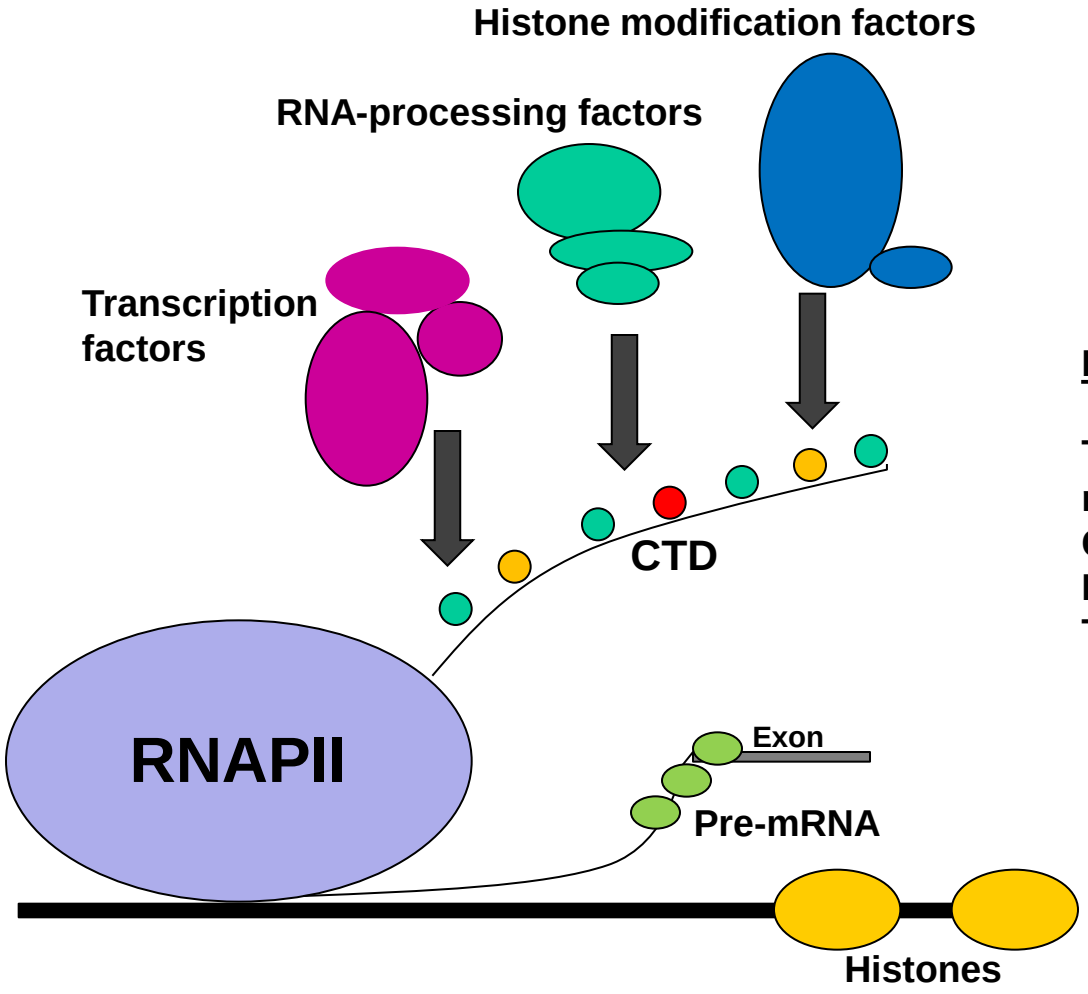


Cdk9 phosphorylates Serine (Ser) in the position 2
Cdk7 phosphorylates Serine (Ser) in the position 5

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



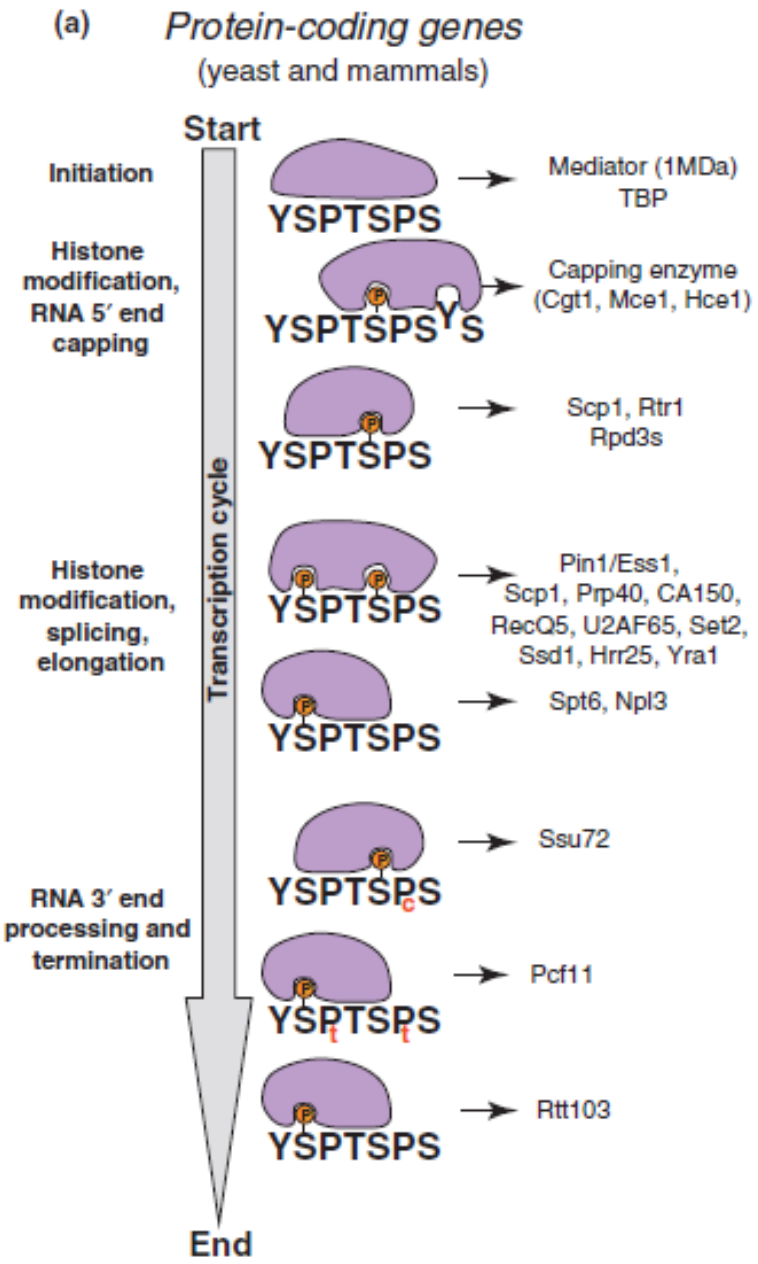
Modified CTD is a binding platform for transcription factors, RNA-processing factors and histone modification factors (code readers)



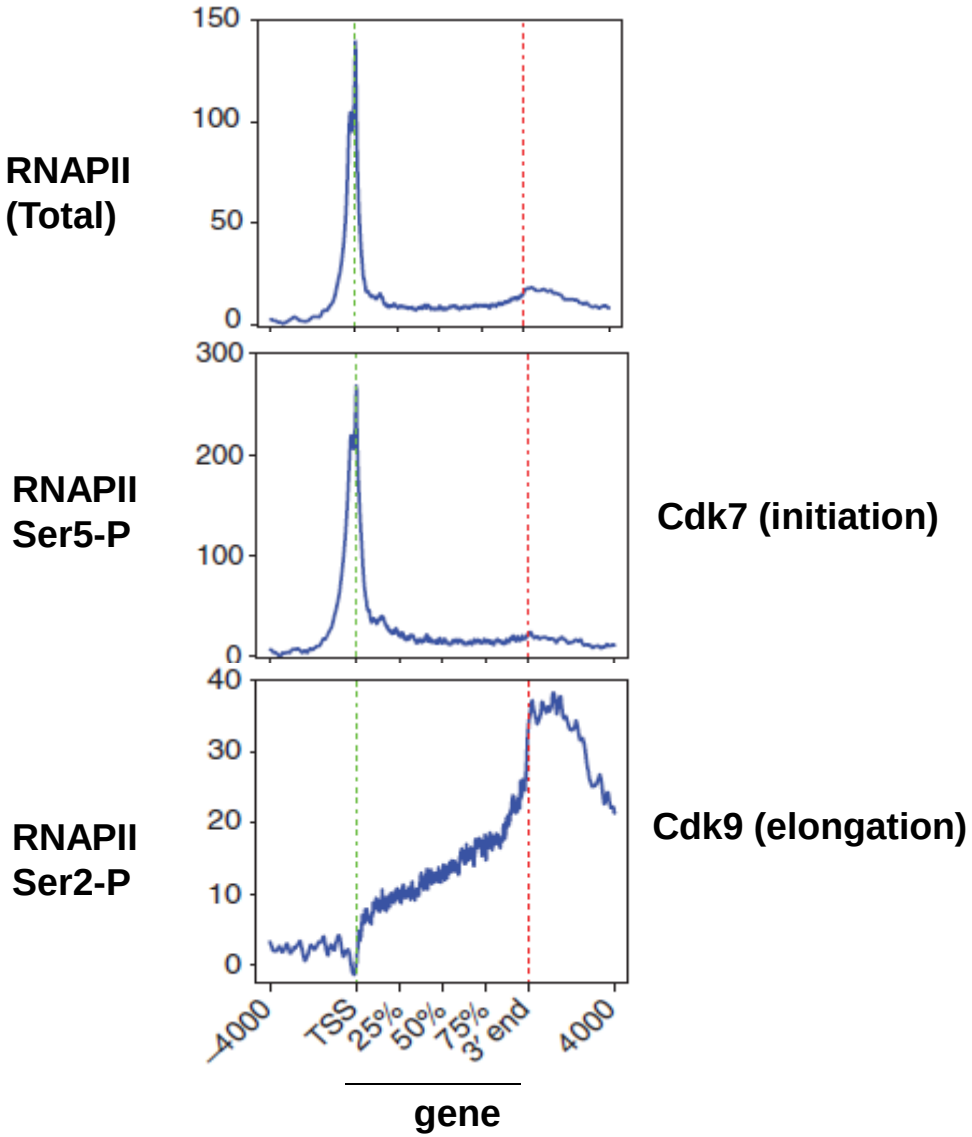
<u>Phosphorylation of the CTD mediates</u>:

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

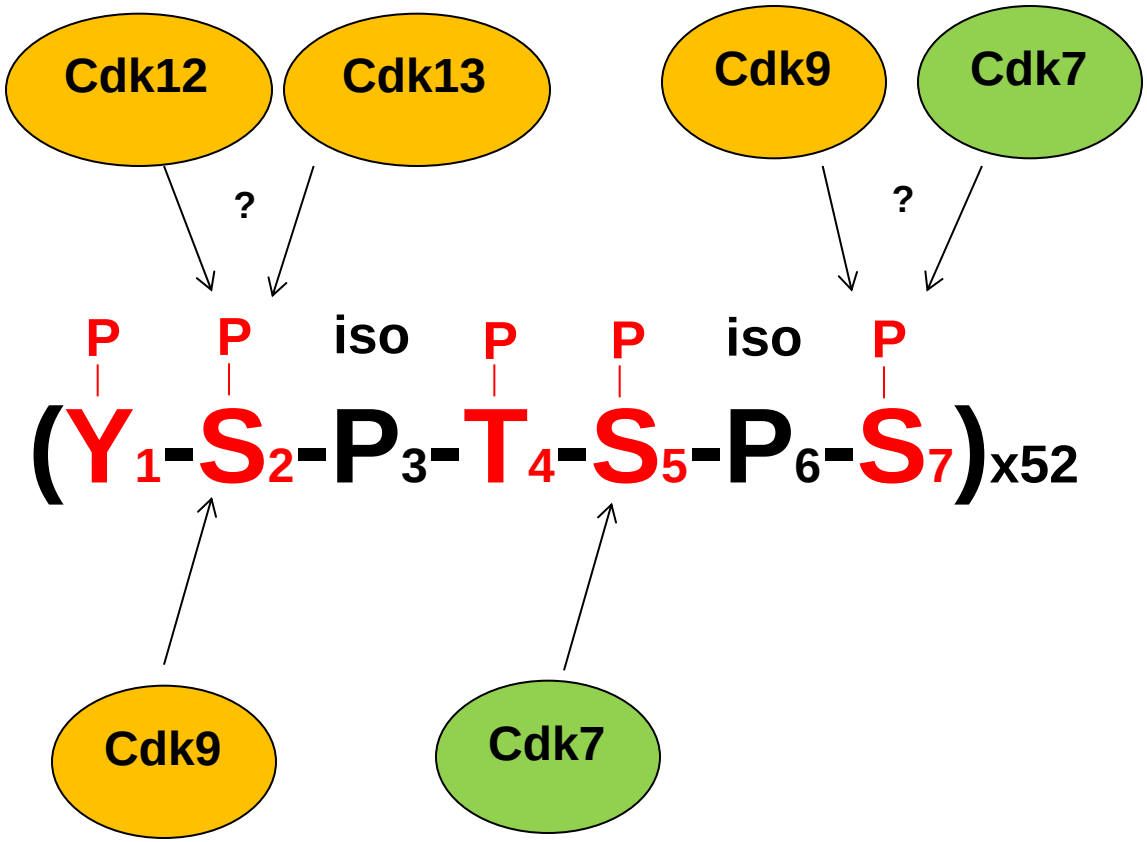
CTD code readers



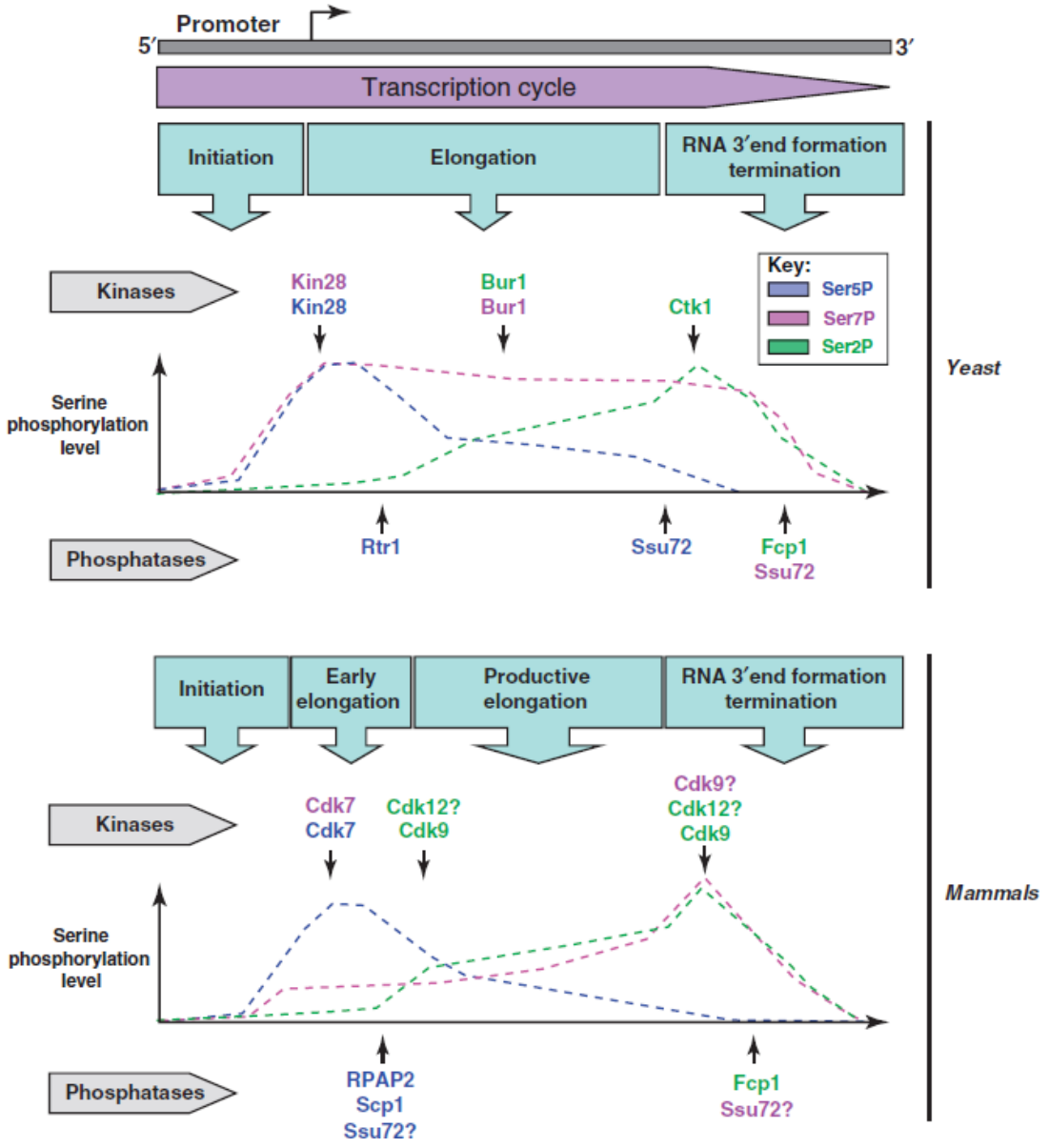
Distribution of phosphorylated Serine 5 and Serine 2 in the CTD of RNAPII along the human protein coding genes



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



Cdks and their roles in transcriptional cycle of yeast and human



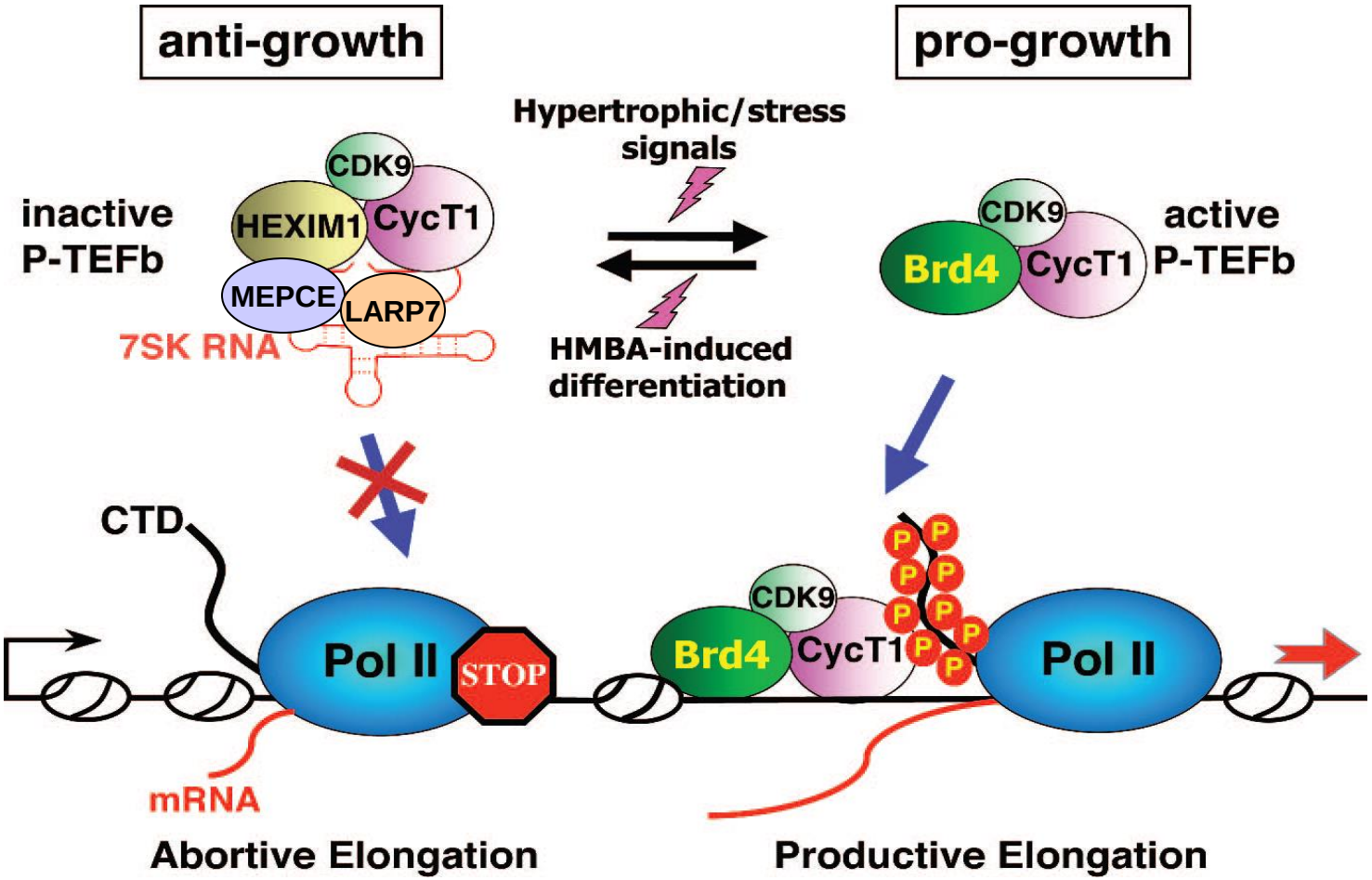
Deregulation of transcription by Cdks leads to the onset of human diseases

-Cancer - aberrant kinase activity of Cdk9 , Cdk12

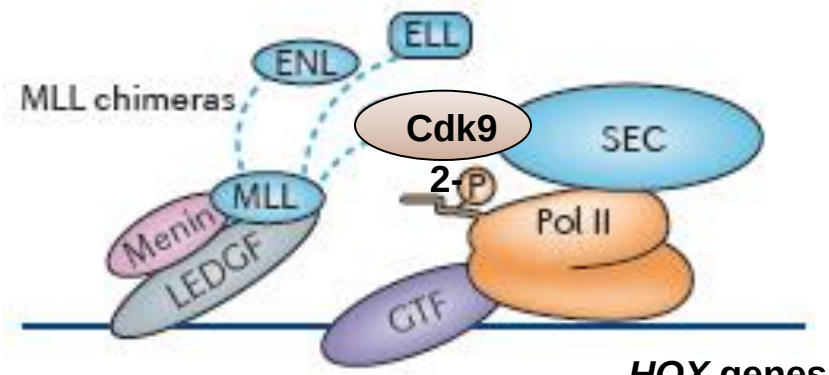
defective transcriptional elongation, mRNA processing

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

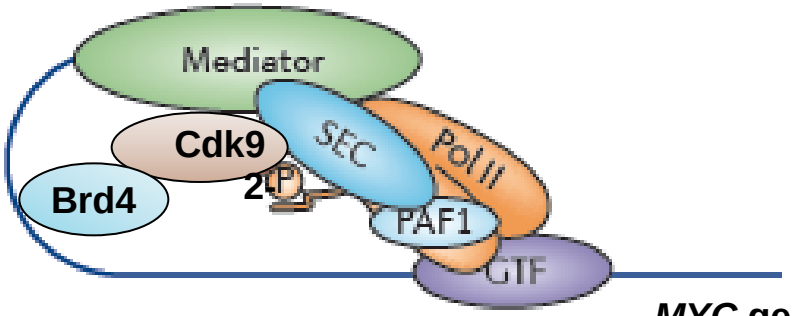
Cdk9 is recruited to most of RNAPII promoters and is present in catalytically active (small) and inactive (large) complexes and regulates transcriptional elongation



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



***HOX* genes**



***MYC* gene**

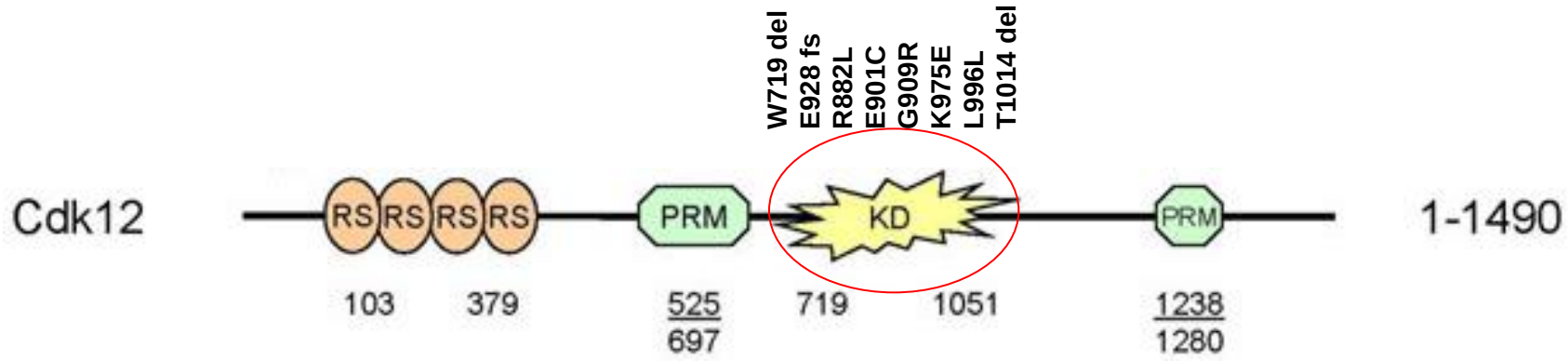
Mixed Lineage Leukemia (MLL)

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

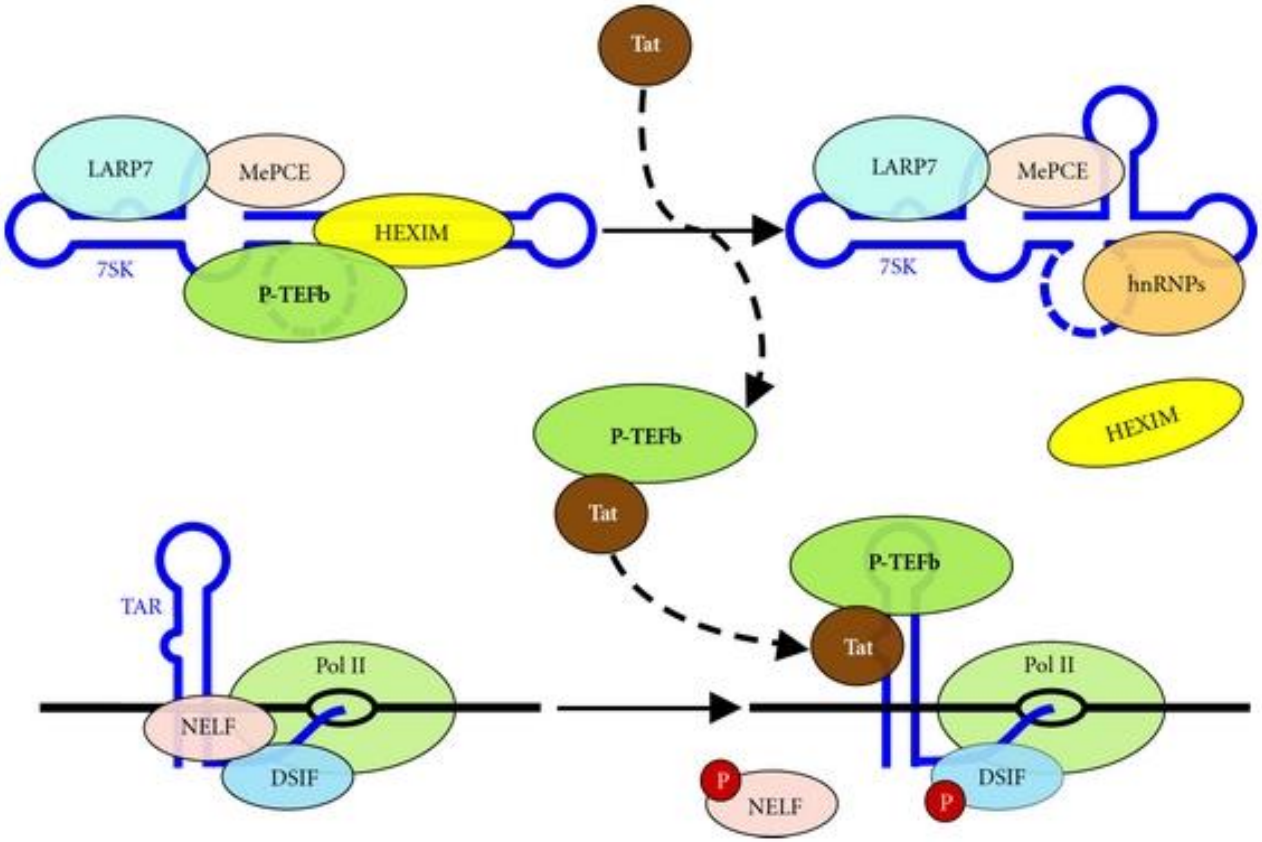


KD=kinase domain

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

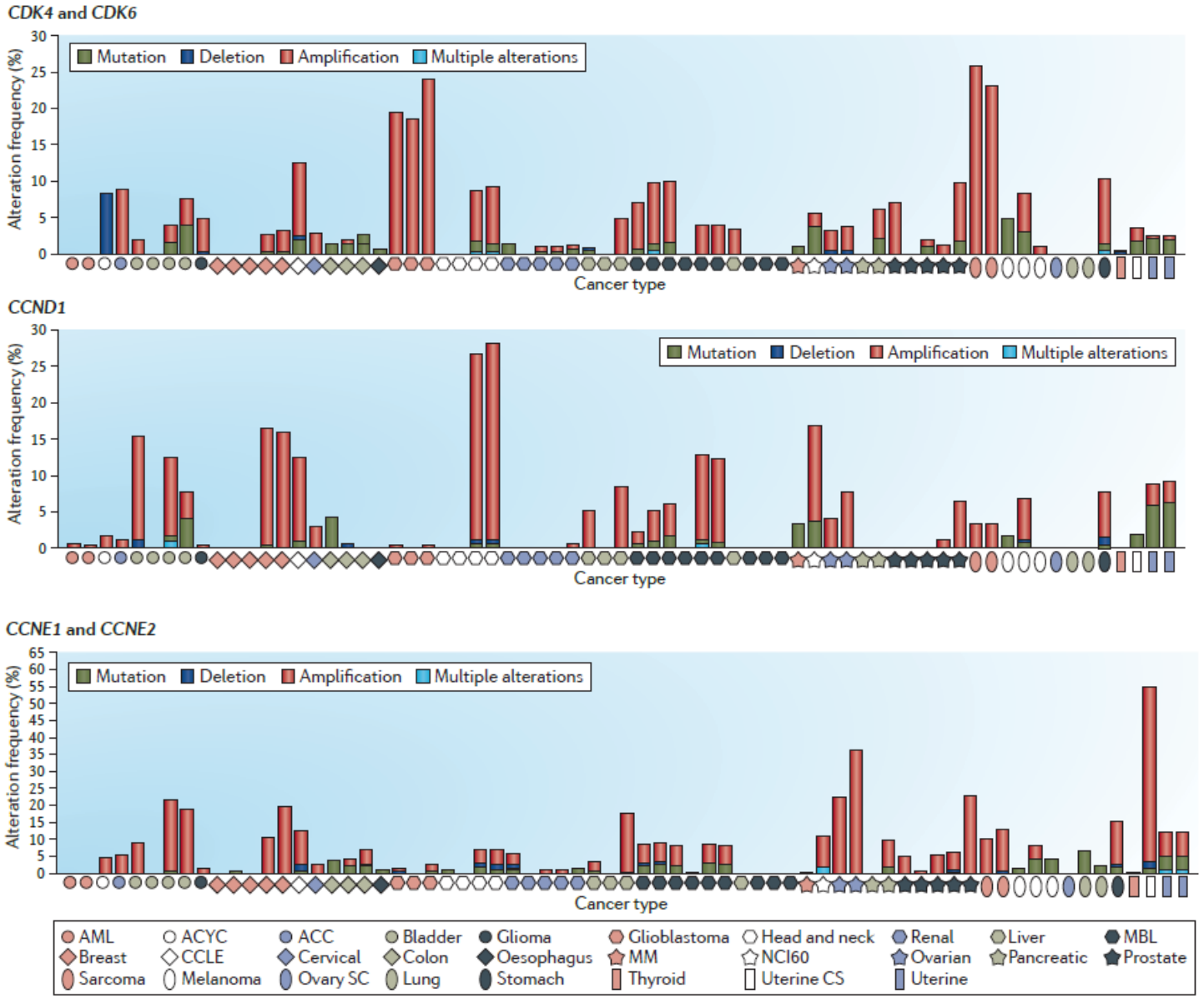
Cdk12 proposed to be a novel tumor suppressor

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

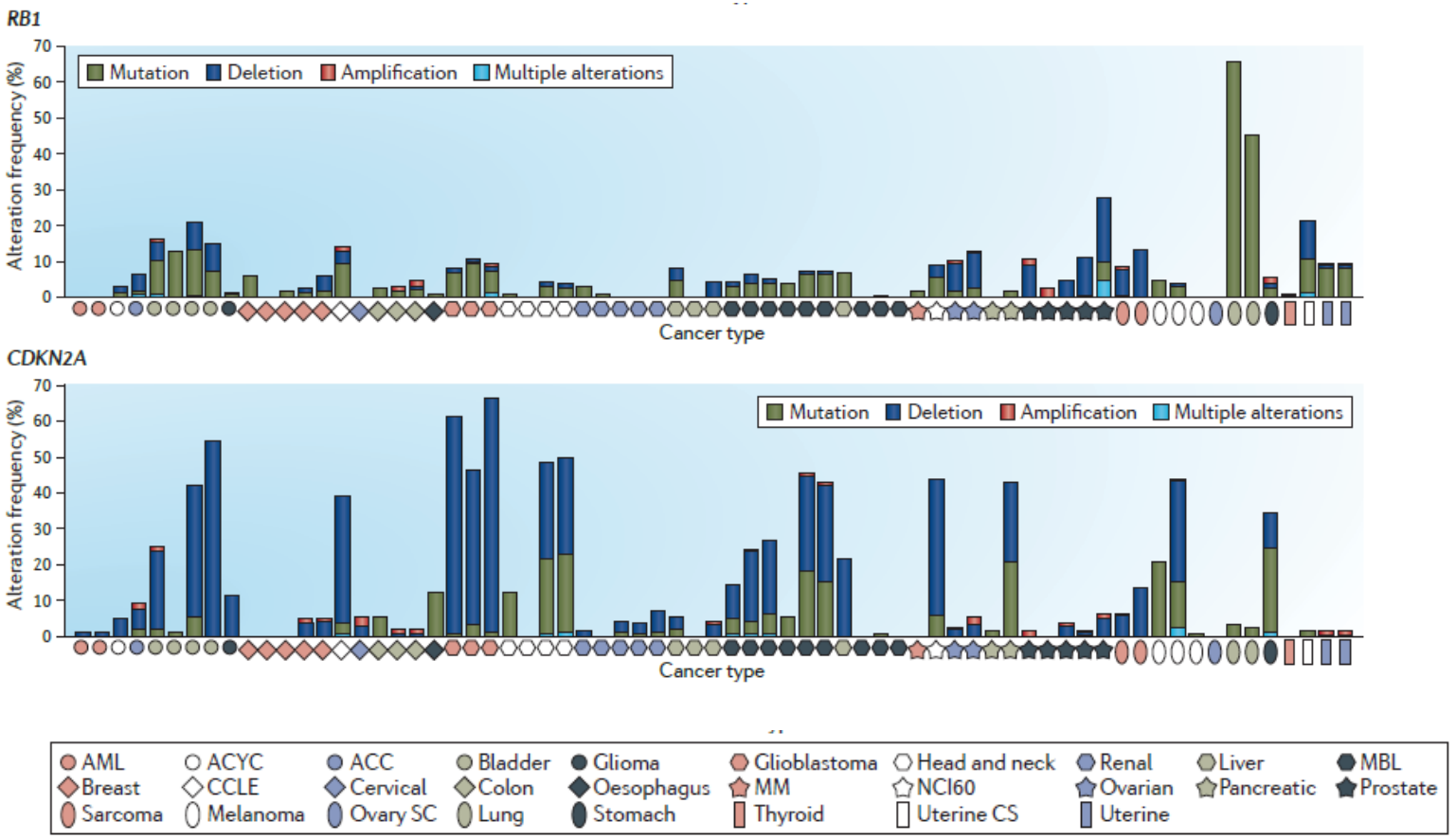


HIV Tat protein "steals" Cdk9 from its complex with inhibitory Hexim1/7SK snRNA; resulting Tat/Cdk9 complex binds to HIV -TAR RNA element and drives HIV transcription in human cells

Inhibition of Cdk activity is a attractive way to treat some diseases (cancer)

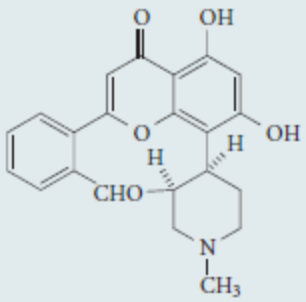


Inhibition of Cdk activity is a attractive way to treat some diseases (cancer)



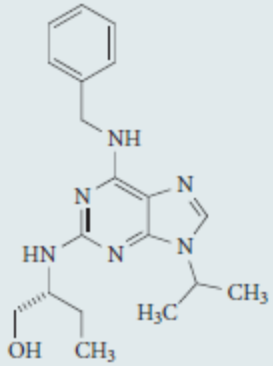
Cell cycle related Cdks/cyclins (Cdk4,Cdk6, CycE, CycD) are often amplified/overexpressed in cancers
Cdk inhibitors (Rb1, Cdkn2n) are often mutated/deleted in various cancers

Cdk inhibitors have low selectivity



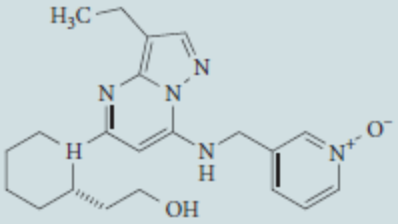
Flavopiridol

IC_{50}
- CDK1: 30 nM
- CDK2: 170 nM
- CDK4: 100 nM
- CDK5: 170 nM
- CDK7: ND
- CDK9: 20 nM



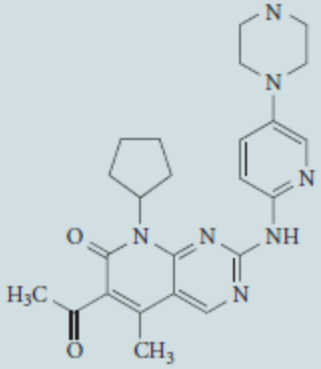
Roscovitine

IC_{50}
- CDK1: 330 nM
- CDK2: 220 nM
- CDK4: >10 μM
- CDK5: 270 nM
- CDK7: 800 nM
- CDK9: 230 nM



Dinaciclib

IC_{50}
- CDK1: 3 nM
- CDK2: 1 nM
- CDK4: ND
- CDK5: 1 nM
- CDK7: ND
- CDK9: 4 nM



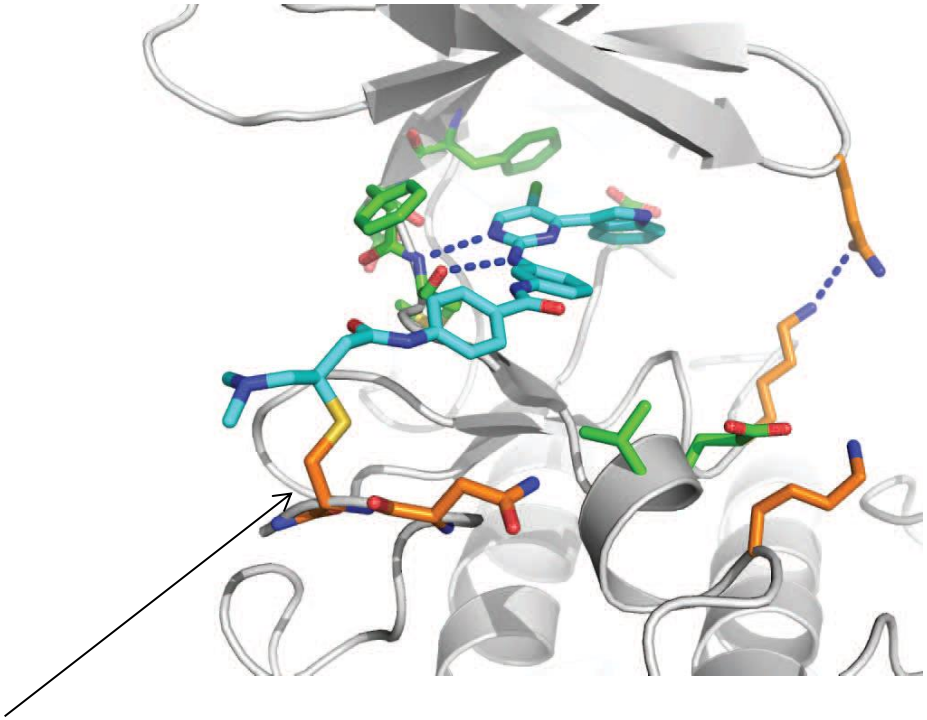
PD-0332991

IC_{50}
- CDK1: >10 μM
- CDK2: >10 μM
- CDK4 : 9–11 nM
- CDK5: >10 μM
- CDK6 : 15 nM
- CDK7: ND
- CDK9: ND

Cdk inhibitors always inhibit many kinases with different efficiency
The outcome depends on inhibition of proper spectrum of kinases in a particular tumor

Recent progress in selectivity of Cdk inhibitors: Covalent inhibitors of Cdk7

```
---PRPICPVETLKEQSNP   323   hsCDK7
---PRPICPVEALKEPANP   323   mCDK7
--------------------   297   hsCDK1
--------------------   298   hsCDK2
---YV----LQRFRH----   305   hsCDK3
--------------------   303   hsCDK4
--------------------   292   hsCDK5
---HLPPS-----QNTSEL   323   hsCDK6
FAGCQIPYPKREFLTEEEP   364   hsCDK8
H--LTSMFEYLAP--PRRK   345   hsCDK9
FPHHRNKRAAP-------A   349   hsCDK10
WPAKSEQQRVK-------R   734   hsCDK11
WQDCHELWSKKRR-RQRQS  1053   hsCDK12
WQDCHELWSKKRR-RQKQM  1031   hsCDK13
---MSSIFTVPNVRLQPEA   429   hsCDK14
---EESLFTVSGVRLKPEM   364   hsCDK15
---TTSIFALKEIQLQKEA   474   hsCDK16
---SVSIFSLKEIQLQKDP   501   hsCDK17
---TASIFSLKEIQLQKDP   483   hsCDK18
FAGCQIPYPKREFLNEDDP   364   hsCDK19
PQRLGGPAPKAHPGPPHIH   313   hsCDK20
```



Cys312 in Cdk7

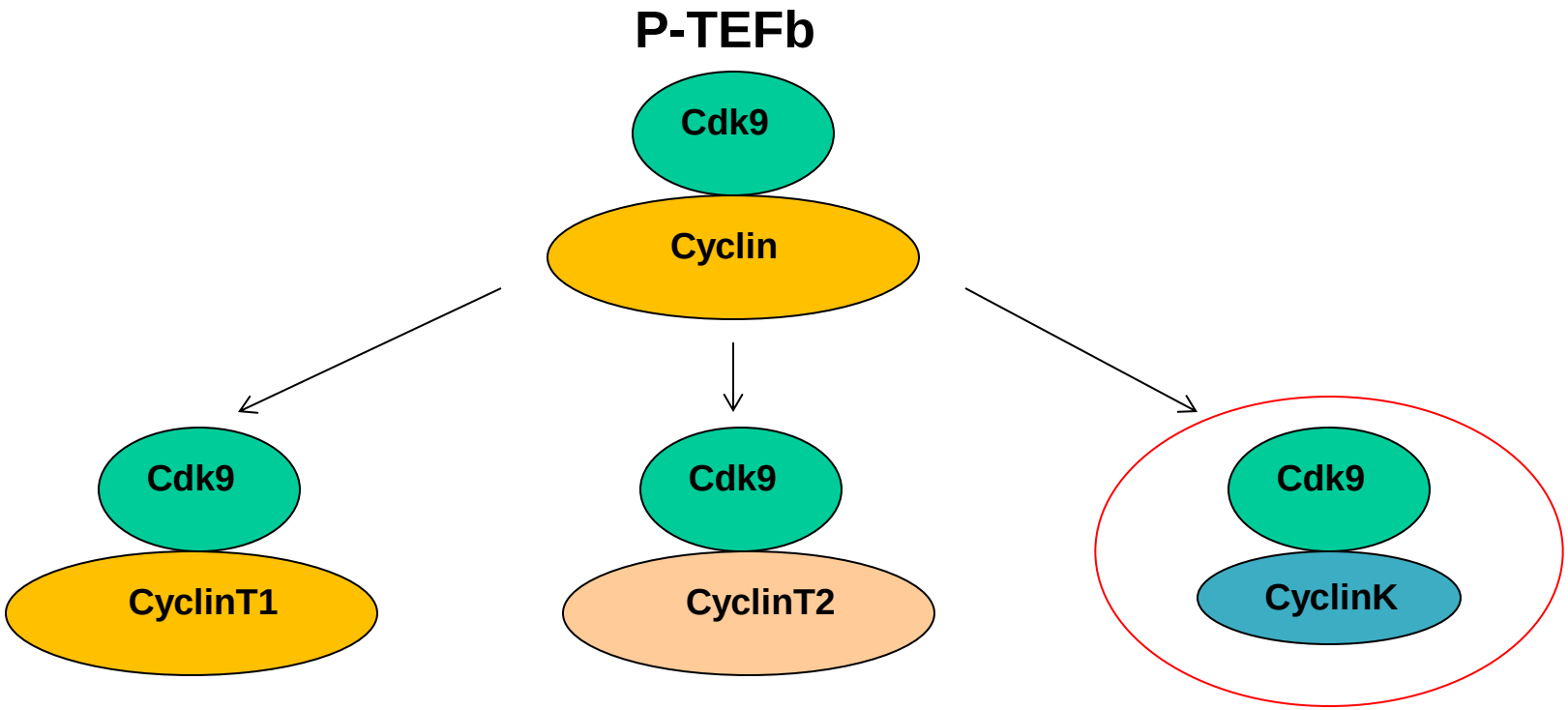
Covalent inhibitor binds cystein residue outside of the kinase domain of Cdk7 and selectively inhibits only Cdk7 kinase activity (similar residue present only in Cdk12 and Cdk13)

Regulation of transcription (gene expression) by cyclin-dependent kinases

Cyclin K/Cdk12-an emerging player in the transcription-coupled genome stability

Role of Cyclin K/Cdk12 in the onset and maintenance of ovarian cancer

Historically, Cdk9 and one of the cyclins (CycT1, CycT2 and CycK) were thought to form positive transcription elongation factor b (P-TEFb)-situation in 2008

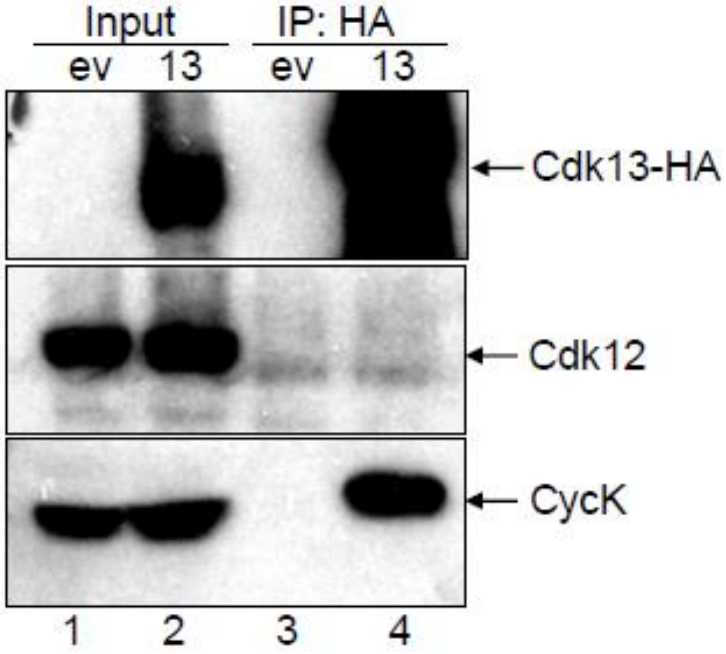


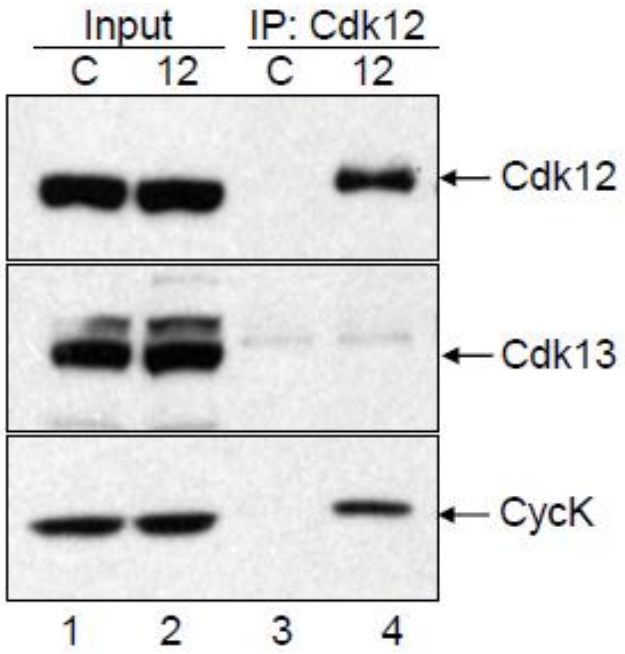
Mostly studied
Supports HIV transcription

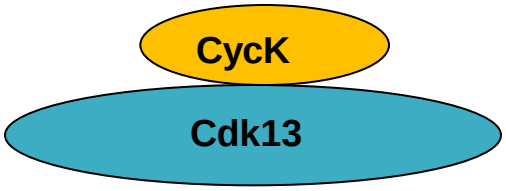
?
Not studied
Does not support HIV transcription

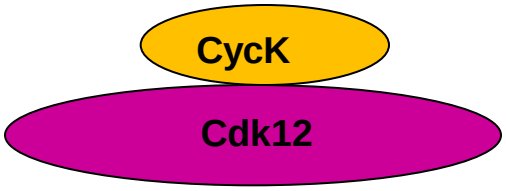
?

CycK binds Cdk12 and Cdk13 in two separate complexes: CycK/Cdk12 and CycK/Cdk13



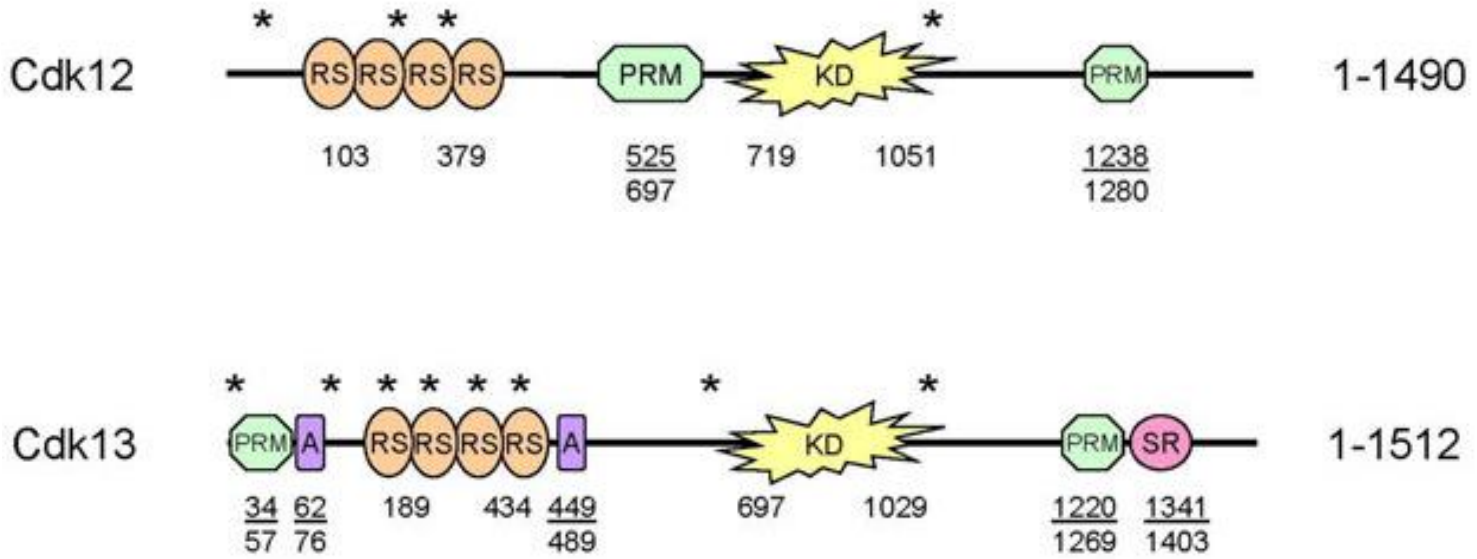


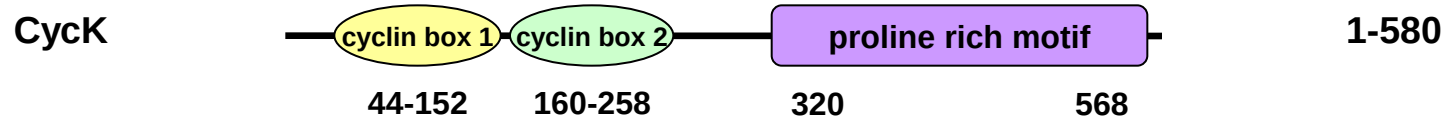




Blazek et al, G&D, 2011

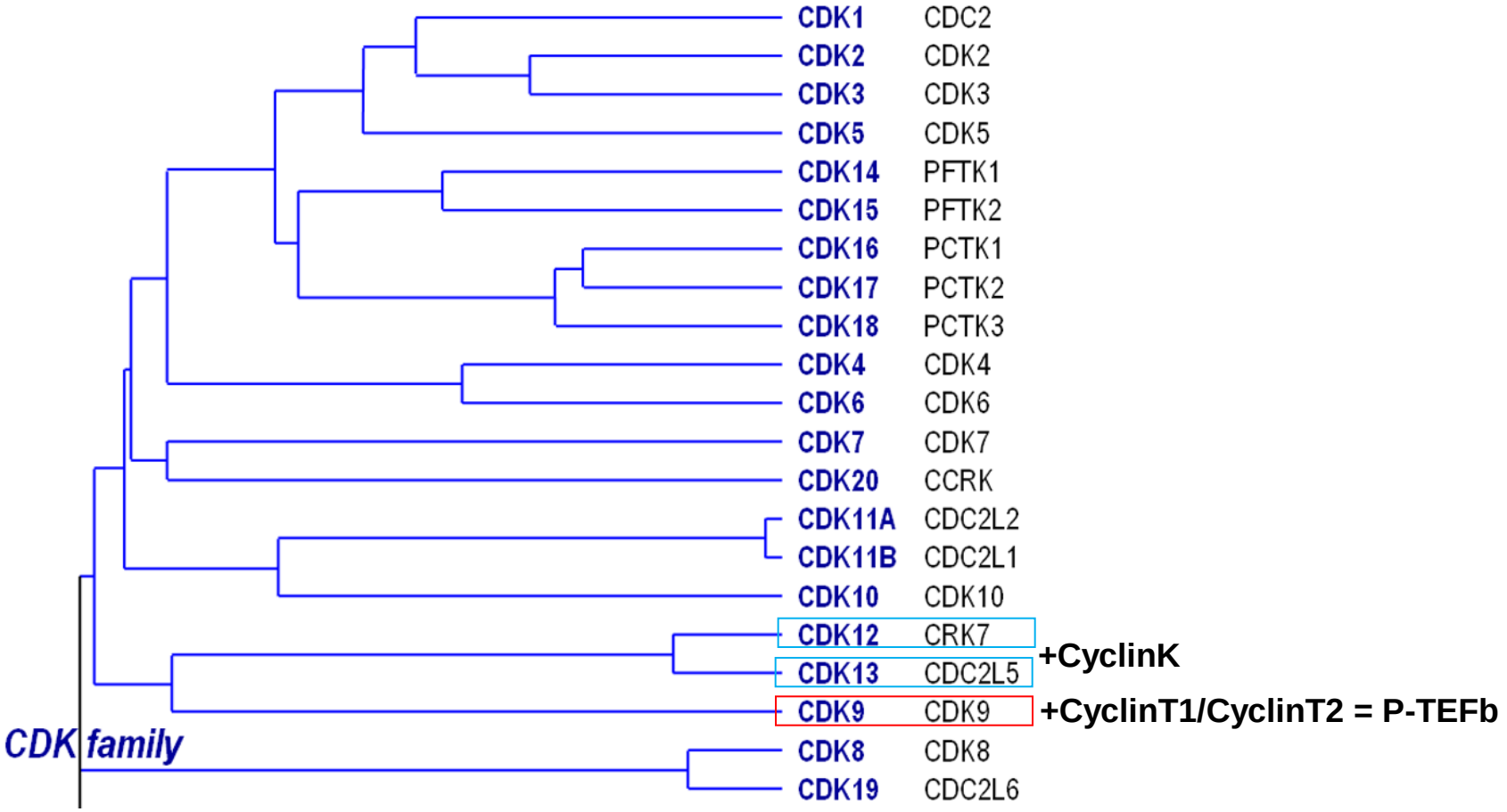
Cdk12 and Cdk13 proteins have similar kinase domains (similarity 93%), but the other domains are different





Kohoutek and Blazek, Cell Div. 2012

Cyclin-dependent kinase (cdk) family (according to similarity of kinase domains)



Adapted according to Malumbres et al., Nature Cell Biol., 2009

Cdk12 is a transcription-associated kinase phosphorylating the C-terminal domain (CTD) of RNA polymerase II (RNAPII)

Genes & Development

CDK12 is a transcription elongation-associated CTD kinase, the metazoan ortholog of yeast Ctk1

Bartlomiej Bartkowiak, Pengda Liu, Hemali P. Phatnani, et al.

Genes Dev. 2010 24: 2303-2316

Genes & Development

The Cyclin K/Cdk12 complex maintains genomic stability via regulation of expression of DNA damage response genes

Dalibor Blazek, Jiri Kohoutek, Koen Bartholomeeusen, et al.

Genes Dev. 2011 25: 2158-2172

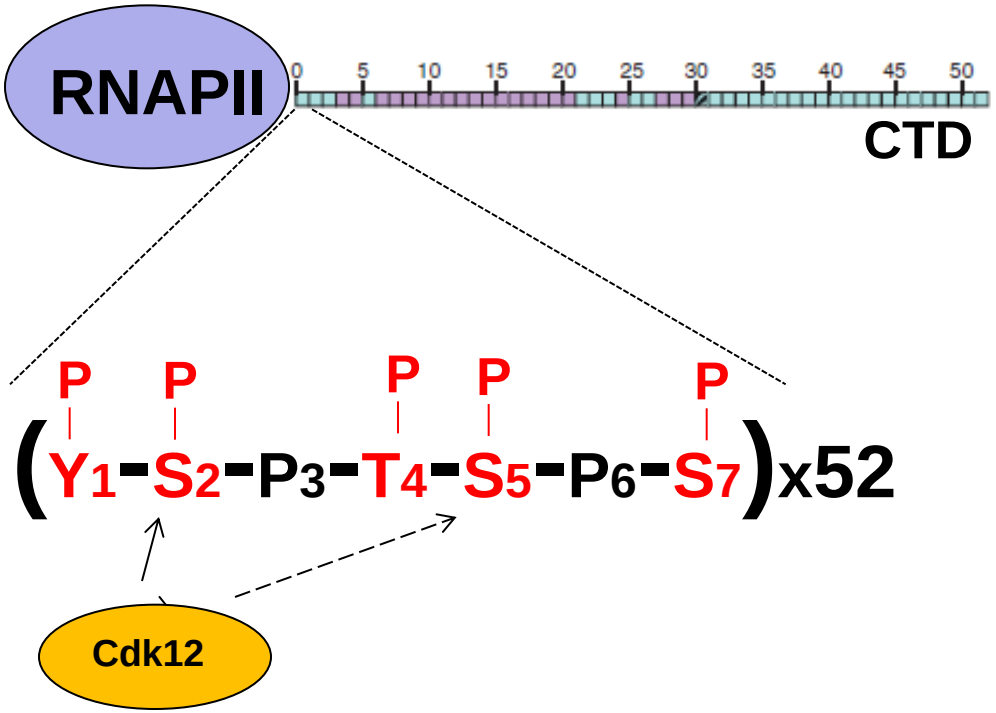
nature COMMUNICATIONS

ARTICLE

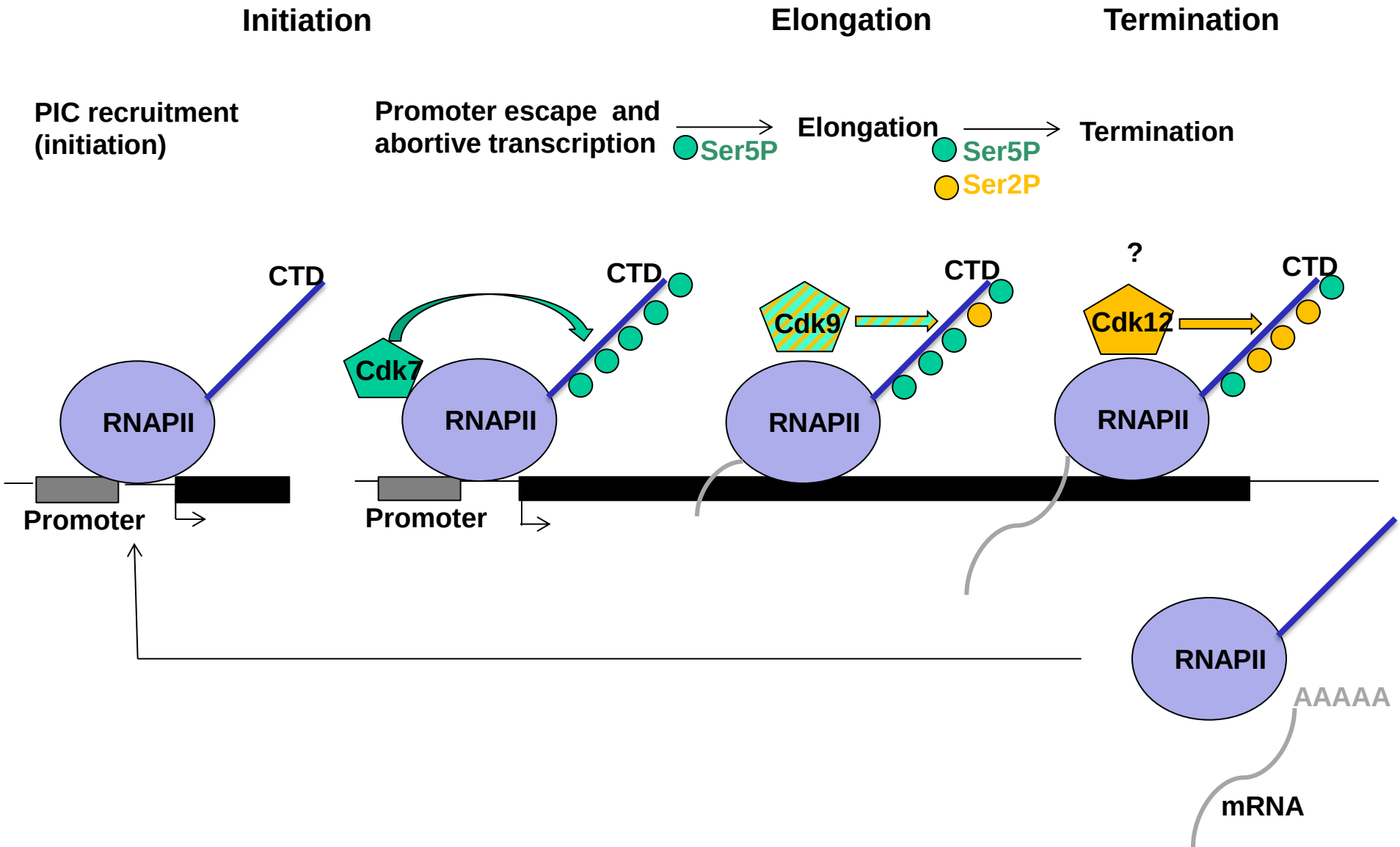
Received 6 Dec 2013 | Accepted 25 Feb 2014 | Published 24 Mar 2014 DOI: 10.1038/ncomms4505 OPEN

The structure and substrate specificity of human Cdk12/Cyclin K

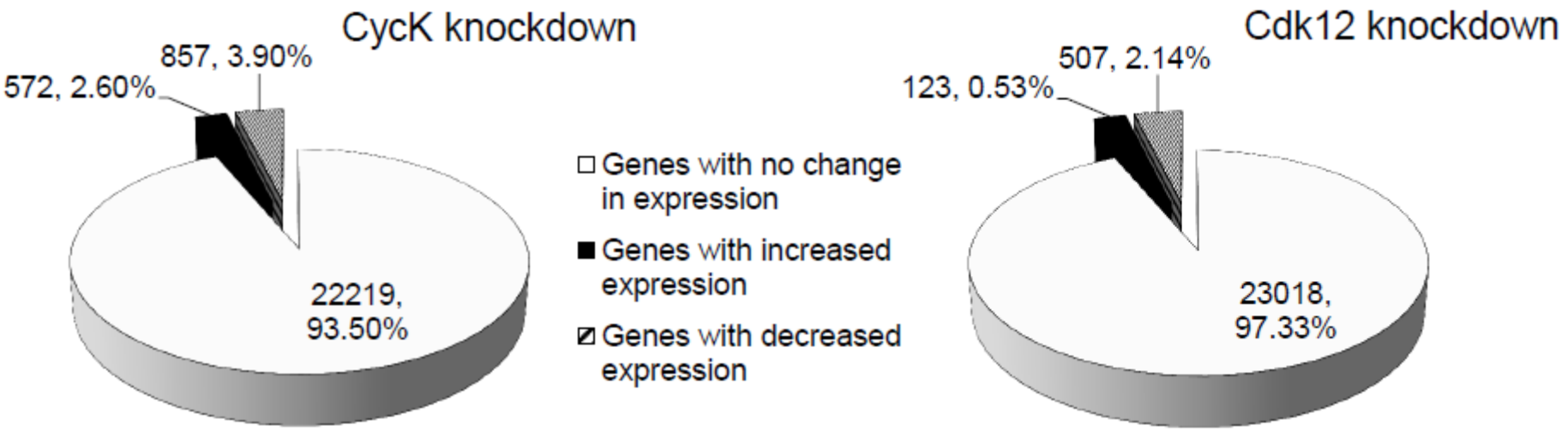
Christian A. Bösken[1,2], Lucas Farnung[2], Corinna Hintermair[3], Miriam Merzel Schachter[4], Karin Vogel-Bachmayr[2], Dalibor Blazek[5], Kanchan Anand[1], Robert P. Fisher[4], Dirk Eick[3] & Matthias Geyer[1,2]

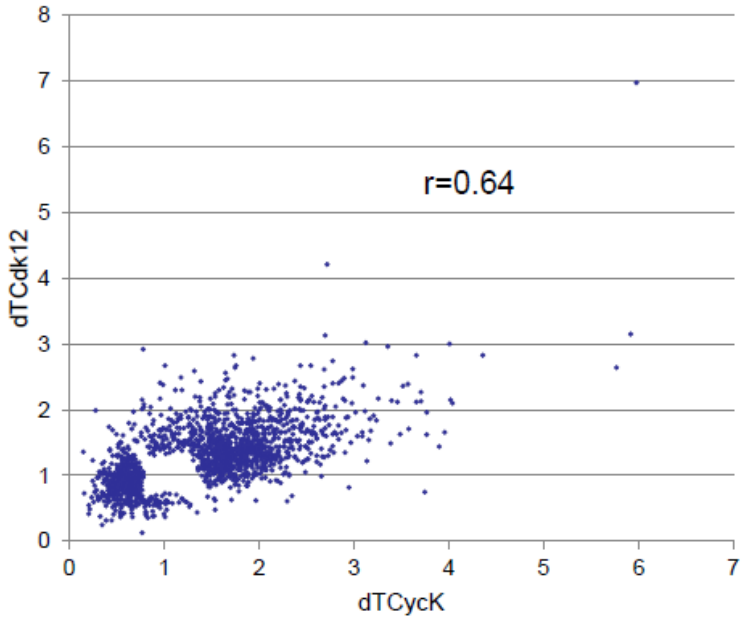


Transcriptional cyclin-dependent kinases phosphorylate the C-terminal domain (CTD) of RNA Polymerase II (RNAPII) and other factors to regulate individual steps of transcription



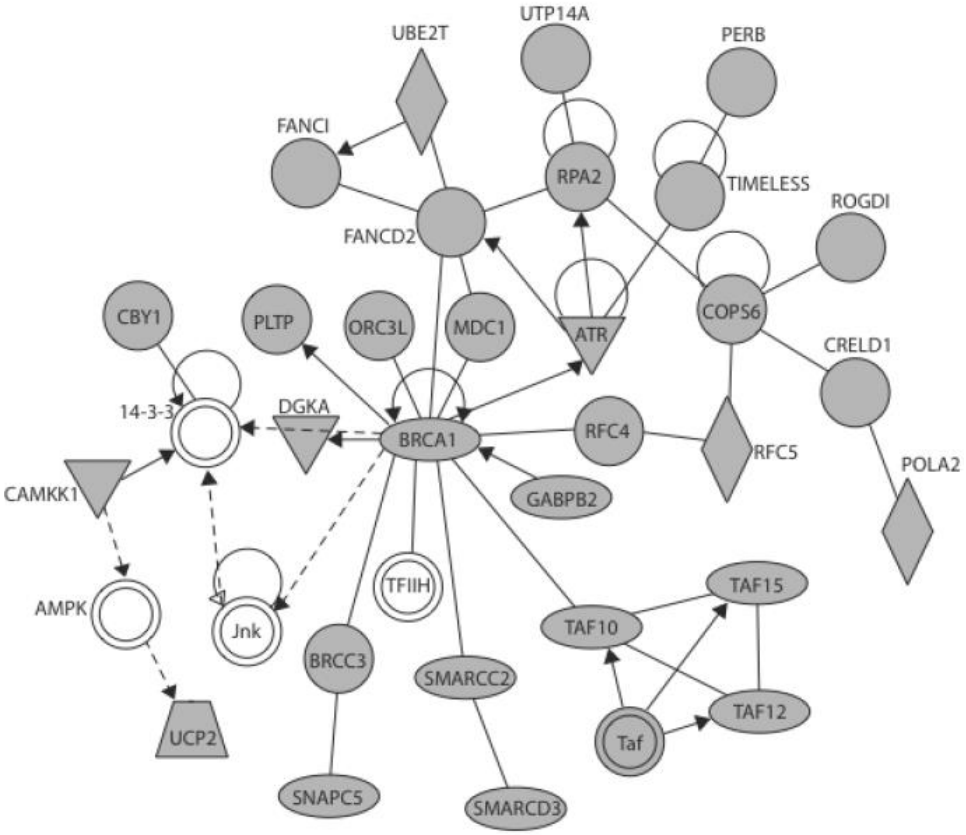
Depletion of CycK/Cdk12 decreases the expression of a small subset of genes

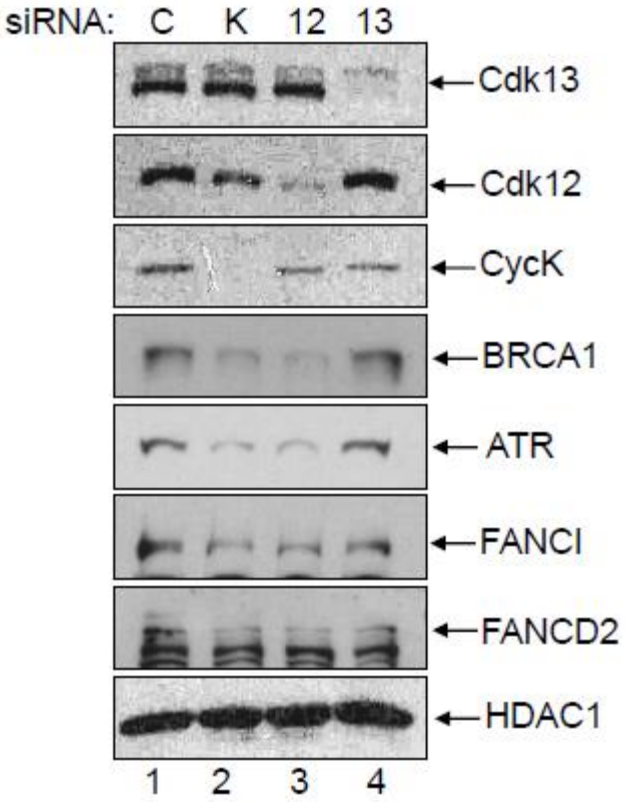




Blazek et al, G&D, 2011

Depletion of CycK/Cdk12 changes the expression of crucial DNA damage response genes

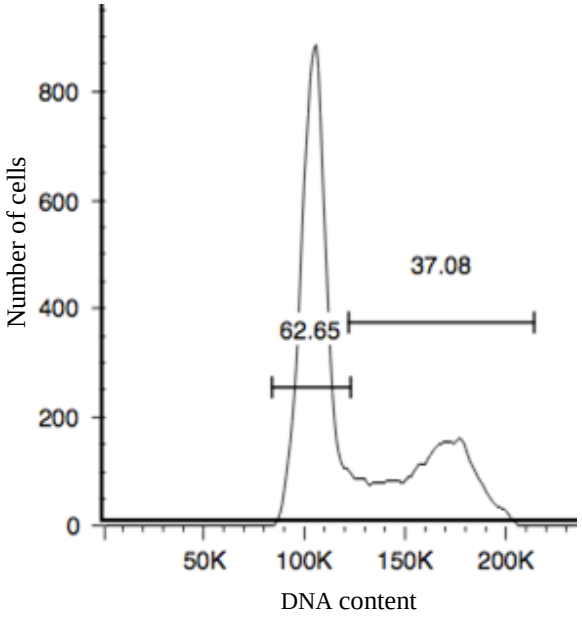




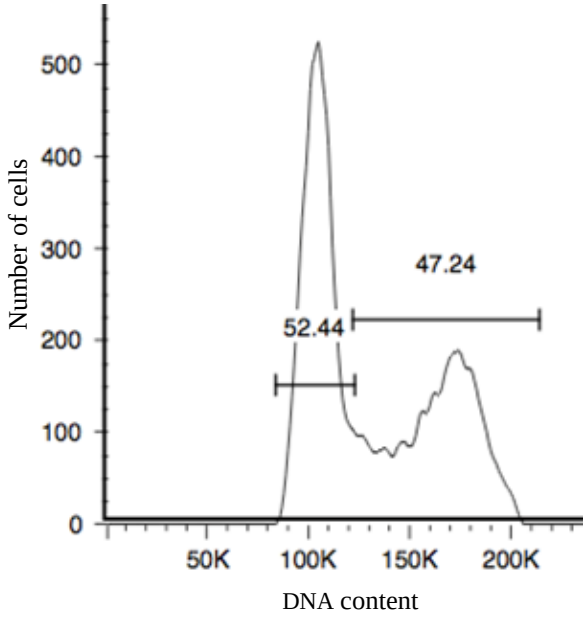
Blazek et al, G&D, 2011

……and depletion of CycK/Cdk12 leads to accumulation of cells in G2/M phase of cell cycle

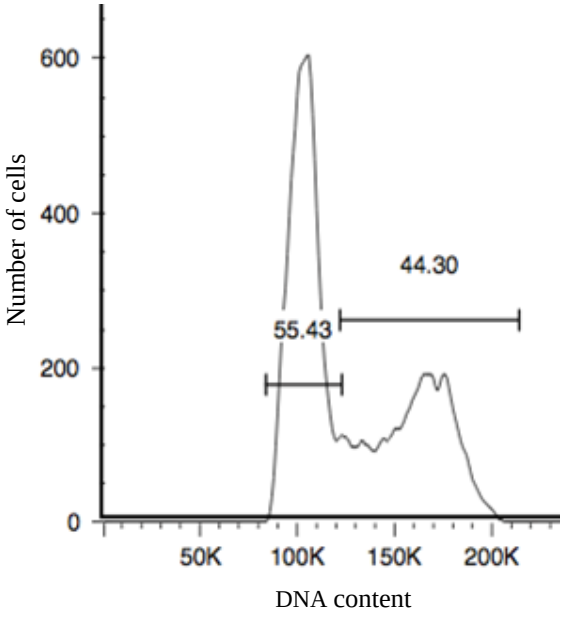
siRNA C



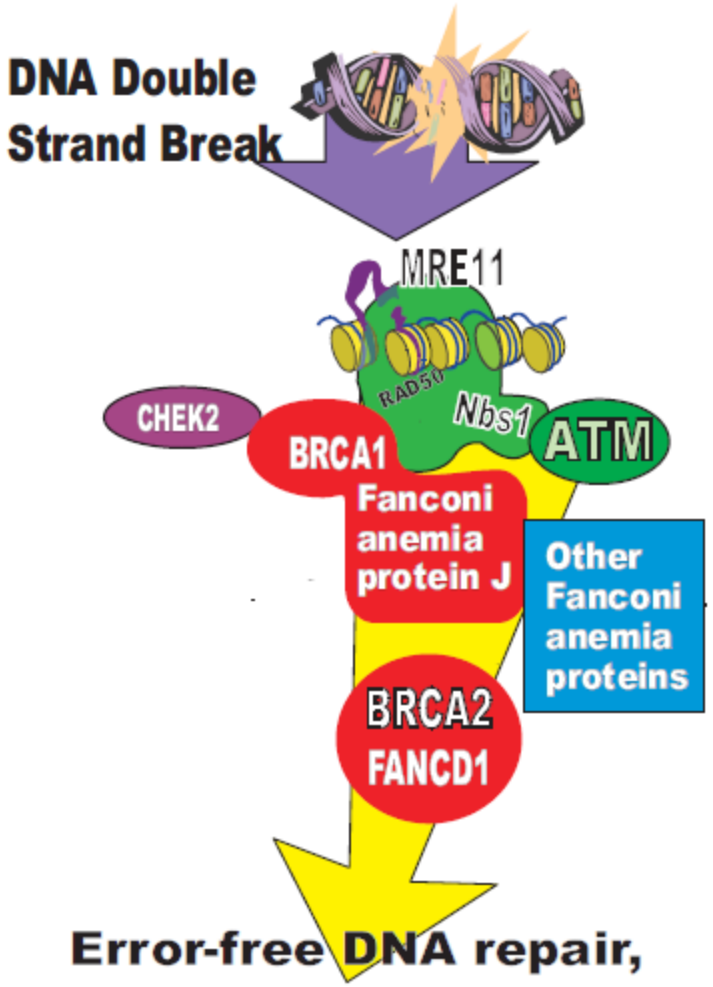
siRNA CycK



siRNA Cdk12



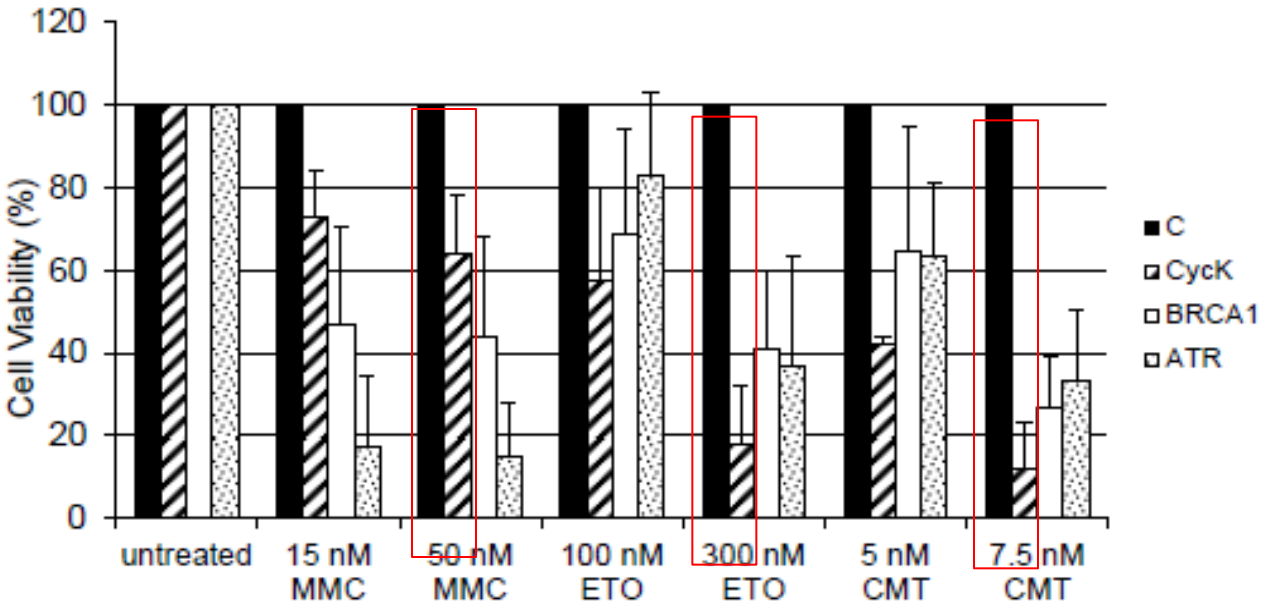
BRCA1, Fanconi anemia proteins, ATR-guardians of genome stability



Maintenance of genome stability

Friedenson, BMC cancer 2007

Loss of CycK/Cdk12 causes sensitivity of cells to a variety of DNA damage agents



Blazek et al, G&D, 2011

Conclusion I

CycK- binds Cdk12 and Cdk13, but not Cdk9

Cdk12 - is a major Ser2 kinase in the CTD of RNAPII

- **directs expression of a small subset of genes**
- **regulates optimal expression of DNA damage response genes (BRCA1, ATR, FANCI, FANCD2)**
- **is crucial for the maintenance of genome stability**
- **candidate tumor suppressor gene**

Cdk12 was found among the most often somatically mutated genes in HGSOC

Integrated genomic analyses of ovarian carcinoma

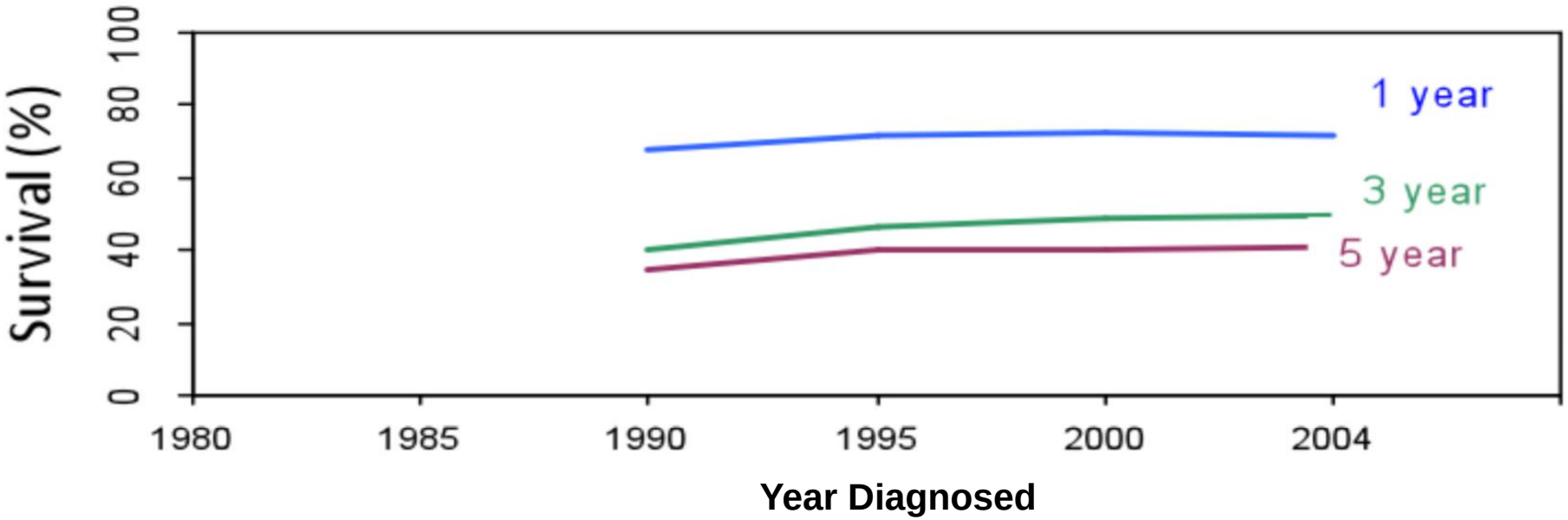
The Cancer Genome Atlas Research Network*

A catalogue of molecular aberrations that cause ovarian cancer is critical for developing and deploying therapies that will improve patients' lives. The Cancer Genome Atlas project has analysed messenger RNA expression, microRNA expression, promoter methylation and DNA copy number in 489 high-grade serous ovarian adenocarcinomas and the DNA sequences of exons from coding genes in 316 of these tumours. Here we report that high-grade serous ovarian cancer is characterized by *TP53* mutations in almost all tumours (96%); low prevalence but statistically recurrent somatic mutations in nine further genes including *NF1*, *BRCA1*, *BRCA2*, *RB1* and *CDK12*; 113 significant focal DNA copy number aberrations; and promoter methylation events involving 168 genes. Analyses delineated four ovarian cancer transcriptional subtypes, three microRNA subtypes, four promoter methylation subtypes and a transcriptional signature associated with survival duration, and shed new light on the impact that tumours with *BRCA1/2* (*BRCA1* or *BRCA2*) and *CCNE1* aberrations have on survival. Pathway analyses suggested that homologous recombination is defective in about half of the tumours analysed, and that NOTCH and FOXM1 signalling are involved in serous ovarian cancer pathophysiology.

Gene	No. of Somatic Mutations (%)	No. of Pubmed Papers	Function
P53	**302 (96%)**	**63852**	**tumor suppressor**
BRCA1	**11 (3%)**	**9231**	**tumor suppressor**
NF1	**13 (4%)**	**3064**	**tumor suppressor**
CDK12	**9 (3%)**	**27**	**?**
BRCA2	**10 (3%)**	**5793**	**tumor suppressor**
RB1	**6 (2%)**	**2050**	**tumor suppressor**

Ovarian cancer

- **204 000 new cases worldwide**
- **results in 125 000 deaths per year**
- **relatively low incidence rate, but extremely lethal**
- **highest death-to-incidence ratio among cancers**
- **overall five-year survival probability in about 42%**
- **70% of deaths are patients with advanced-stage high-grade serous ovarian carcinoma (HGSOC)**



Vaugham et al., Nat. Rev Cancer, 2011

High-grade serous ovarian carcinoma (HGSOC)

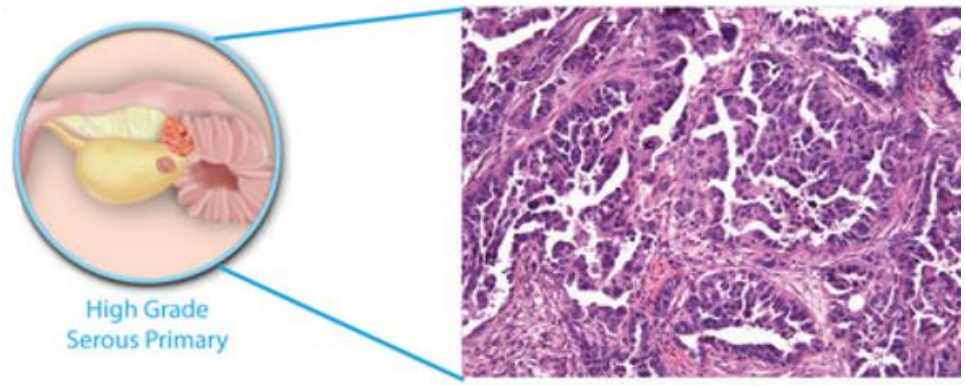
- **Narrow mutational spectrum - p53** mutated in 96% of patients
  - recurrent mutations in eight genes including BRCA1/2

~ 50% of patients have a **defect in homologous recombination (HR)** DNA repair pathway

potentially sensitive to **PARP inhibitors** therapy

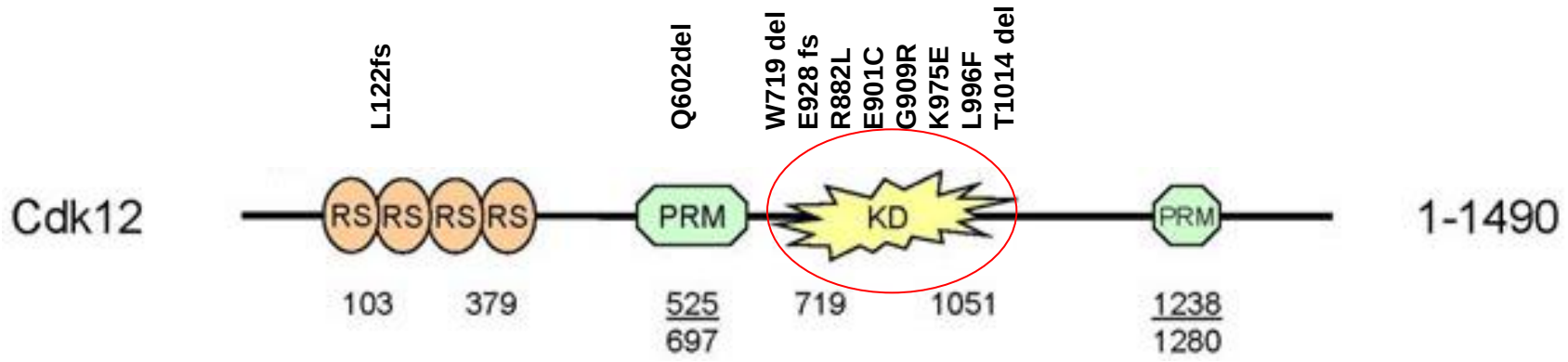
- **Defect in HR - *BRCA1/2* mutations and *BRCA1* epigenetic silencing**
  - Fanconi anemia genes mutations (FANCI, FANCD2, FANCA)
  - Rad family genes mutations
  - DDR genes mutations (*ATR, ATM, Chek1, Chek2*)
  -

What is the role of CDK12????



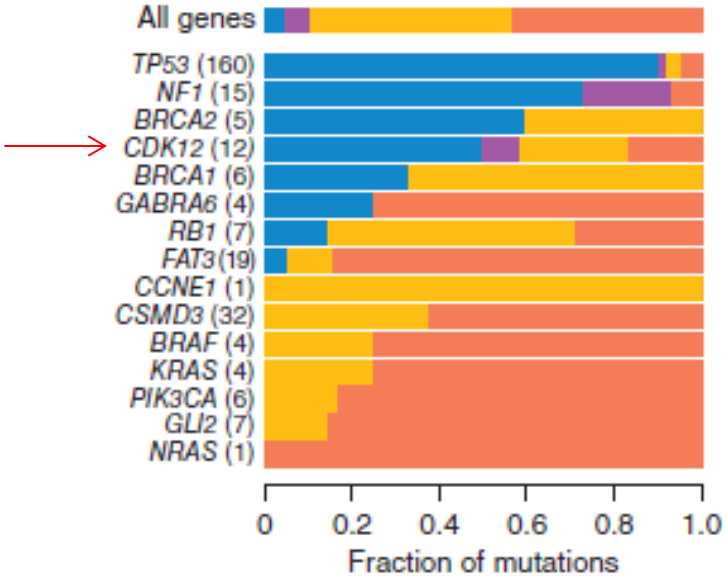
Vaughan et al, Nature Rev Cancer 2012

HGSOC-related mutations in *Cdk12* are clustered in its kinase domain and lead to potential loss of Cdk12 function



KD=Kinase Domain (aa 719-1051)

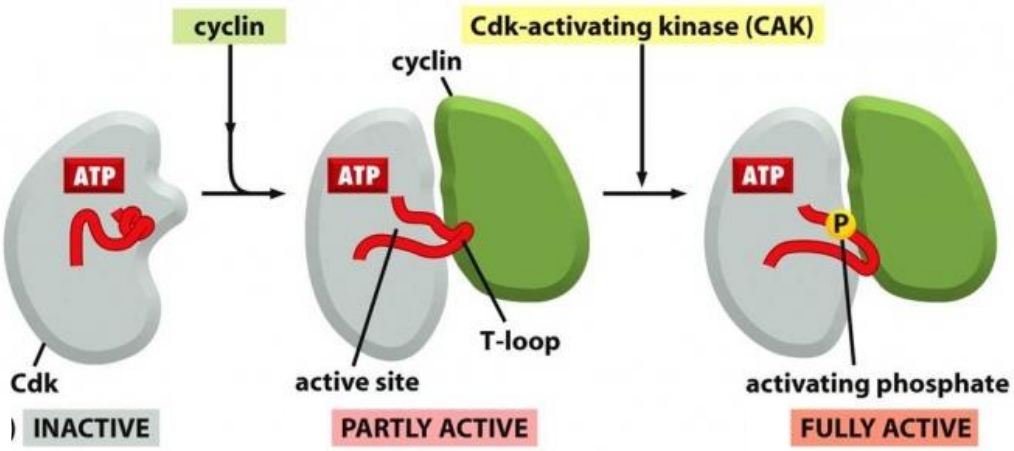
Adapted from Kohoutek and Blazek, Cell Div., 2012



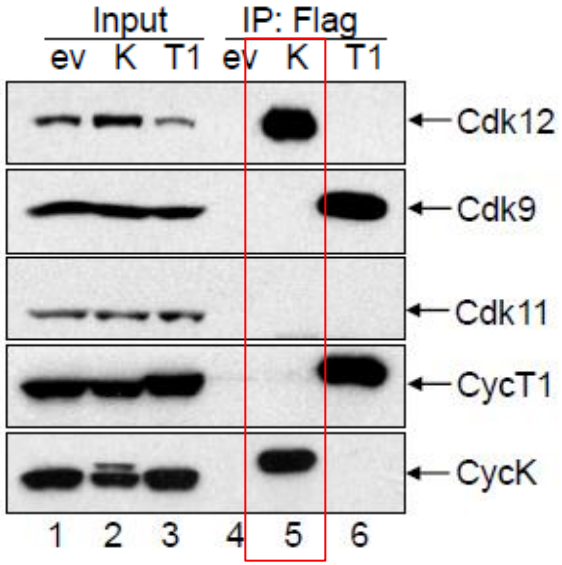
Most of the HGSOC-related *Cdk12* mutations are homozygous

Carter et al, Nature Biotechnology, 2012

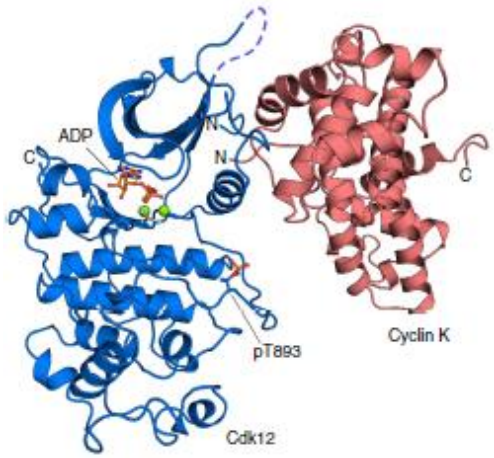
Cdk12 forms a complex with its activating Cyclin, Cyclin K (CycK)



Alberts et al, Mol Biol of Cell, 2002

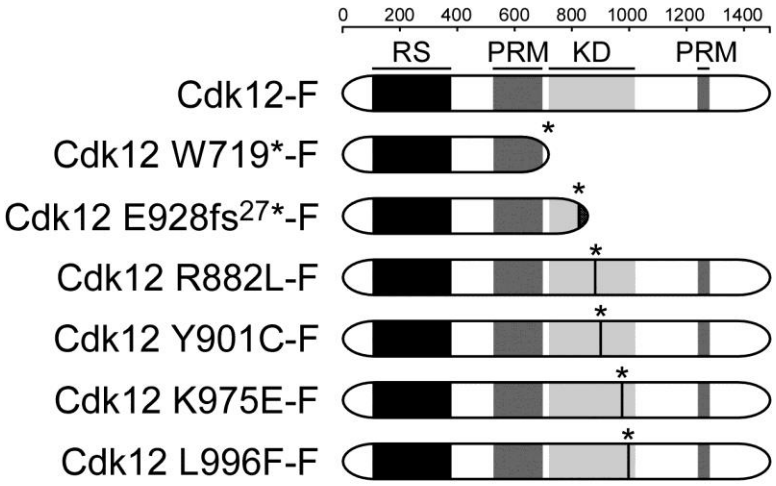


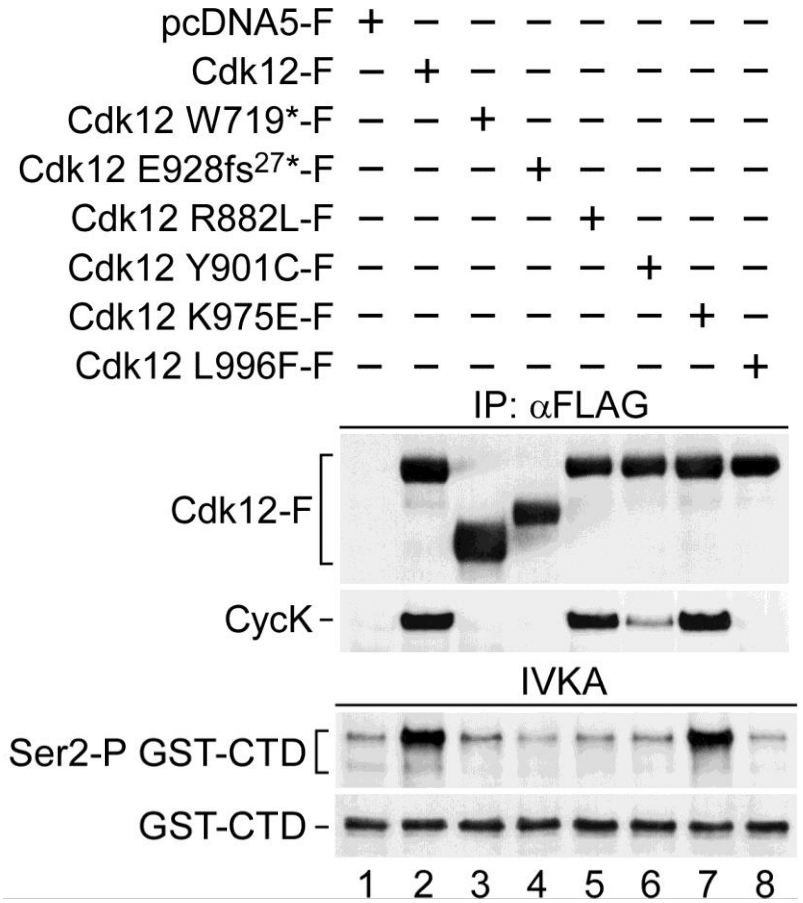
Blazek et al, G&D, 2011
Bartkowiak et al, G&D, 2010



Bosken et al, Nature Comm, 2014

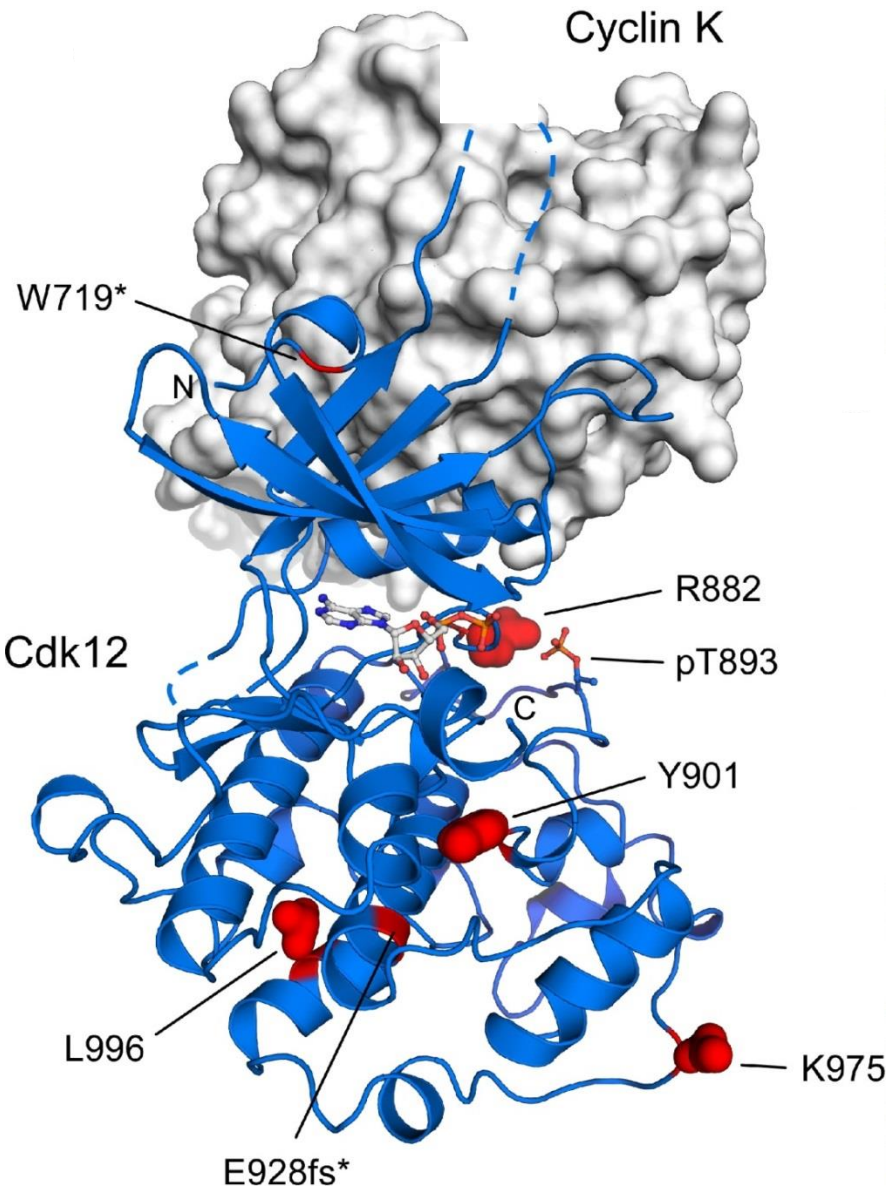
Most of *Cdk12* mutations in HGSOC abrogate the kinase activity of Cdk12 and some lead to defective interaction between CycK and Cdk12

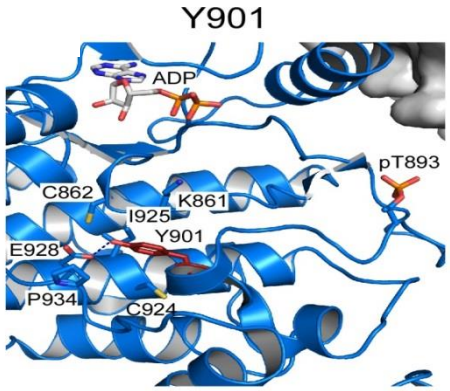


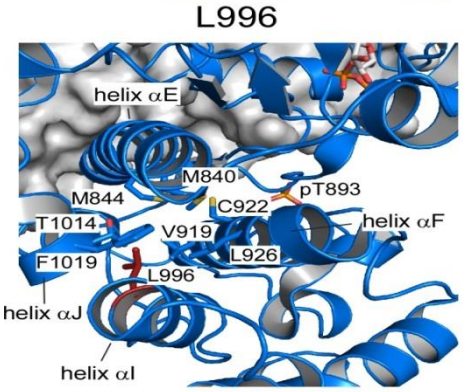


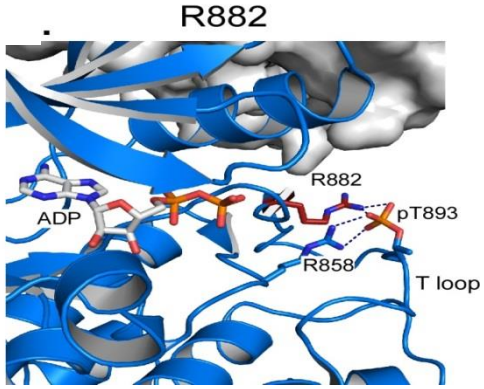
Ekumi, Paculova et al, NAR 2015

Structural insights into the detrimental effects of *Cdk12* mutations on the kinase activity of Cdk12



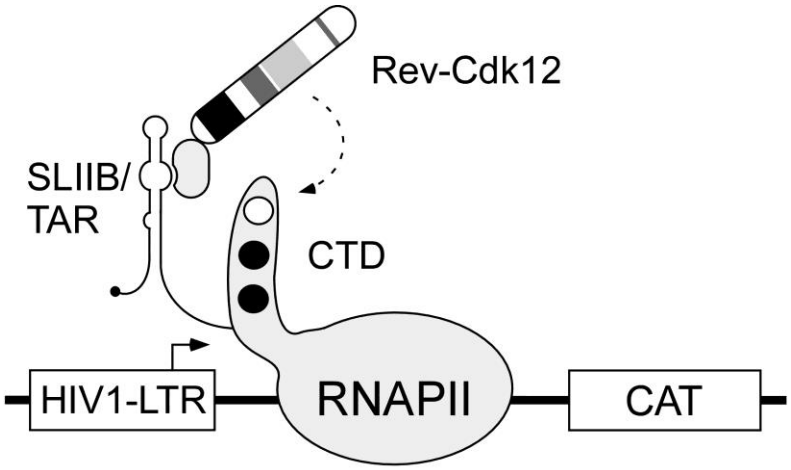


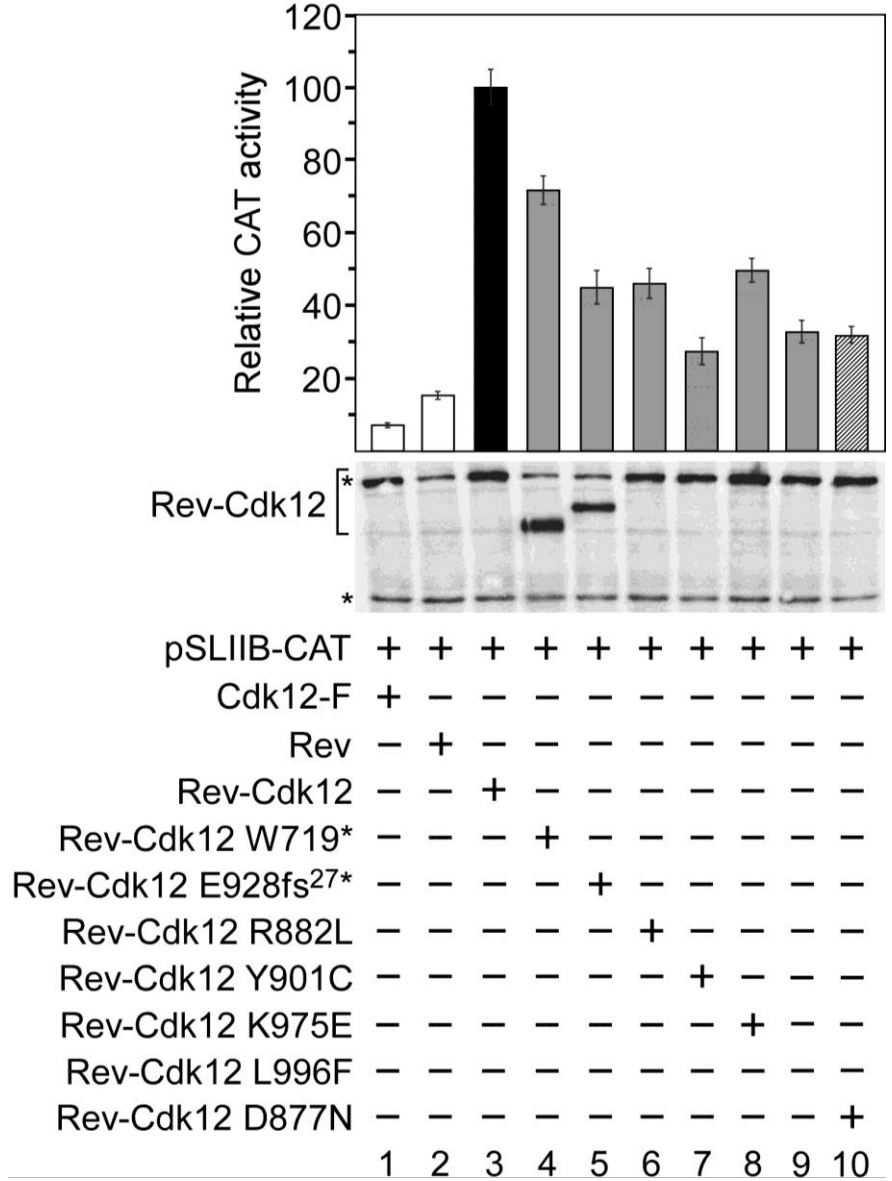




Ekumi, Paculova et al, NAR 2015

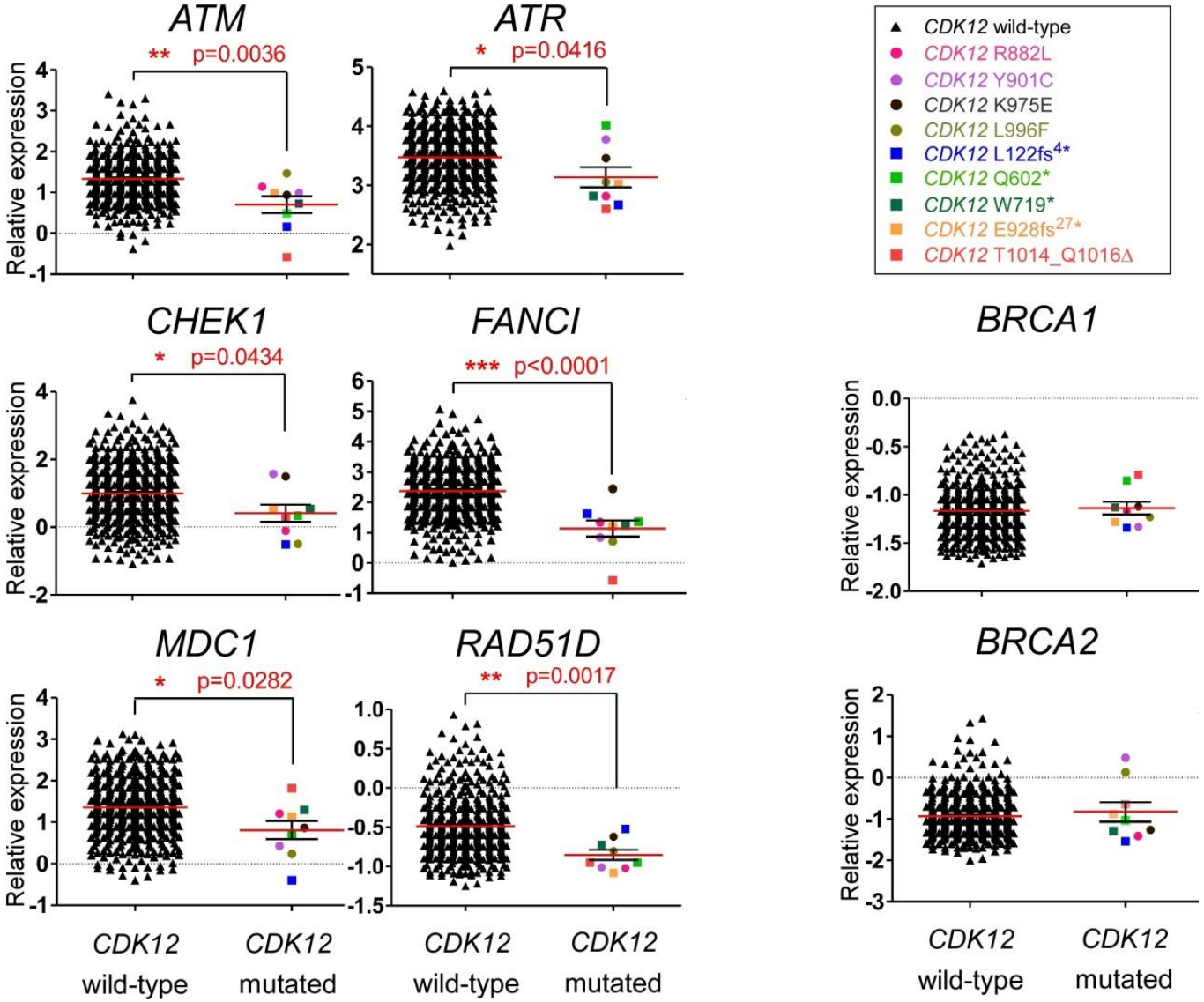
Cdk12 mutations in HGSOC decrease the transcriptional activation by Cdk12 in reporter assay





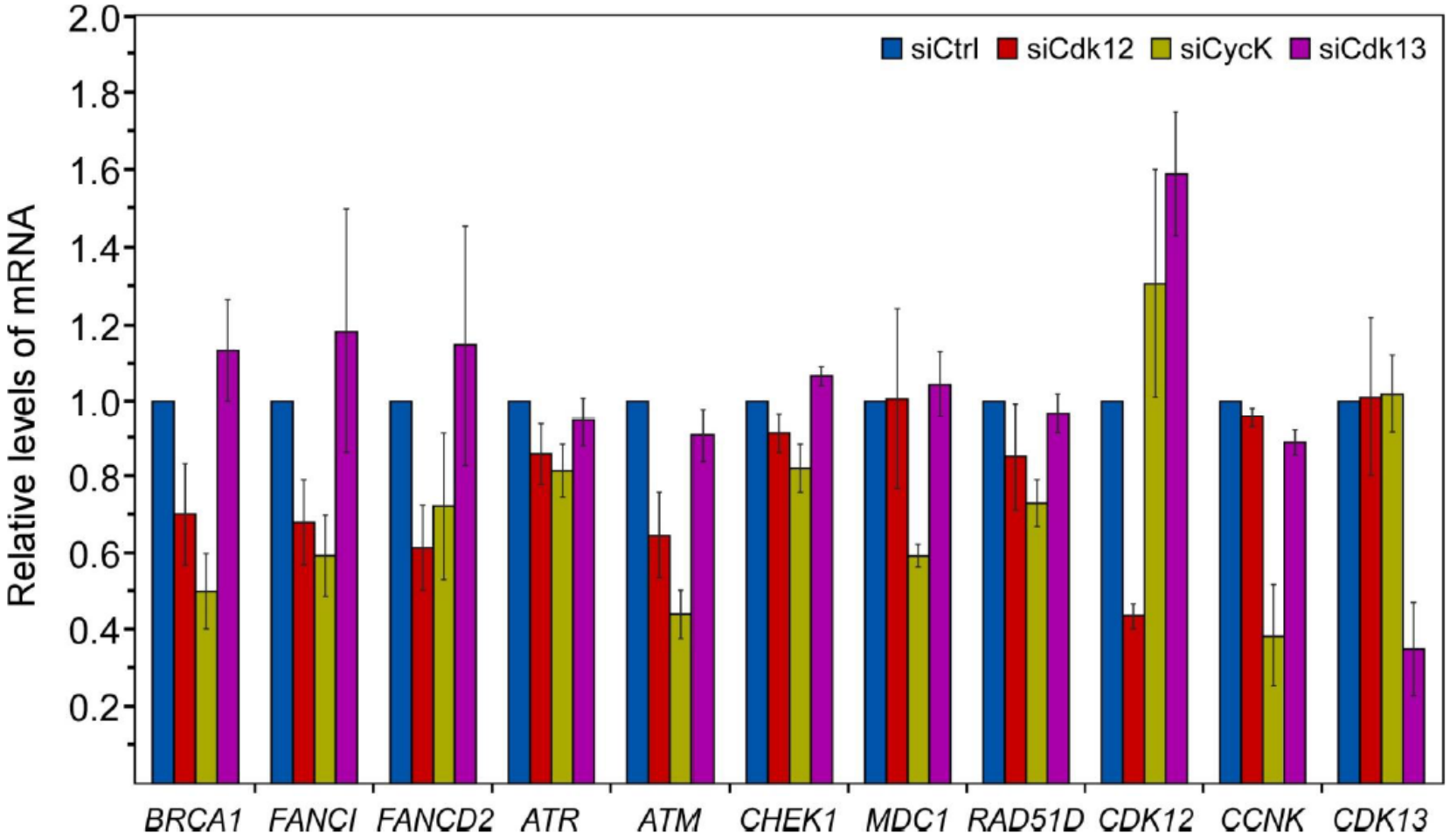
Ekumi, Paculova et al, NAR 2015

Cdk12 mutations in HGSOC patient samples cause down-regulation of genes of the homologous recombination (HR) repair pathway



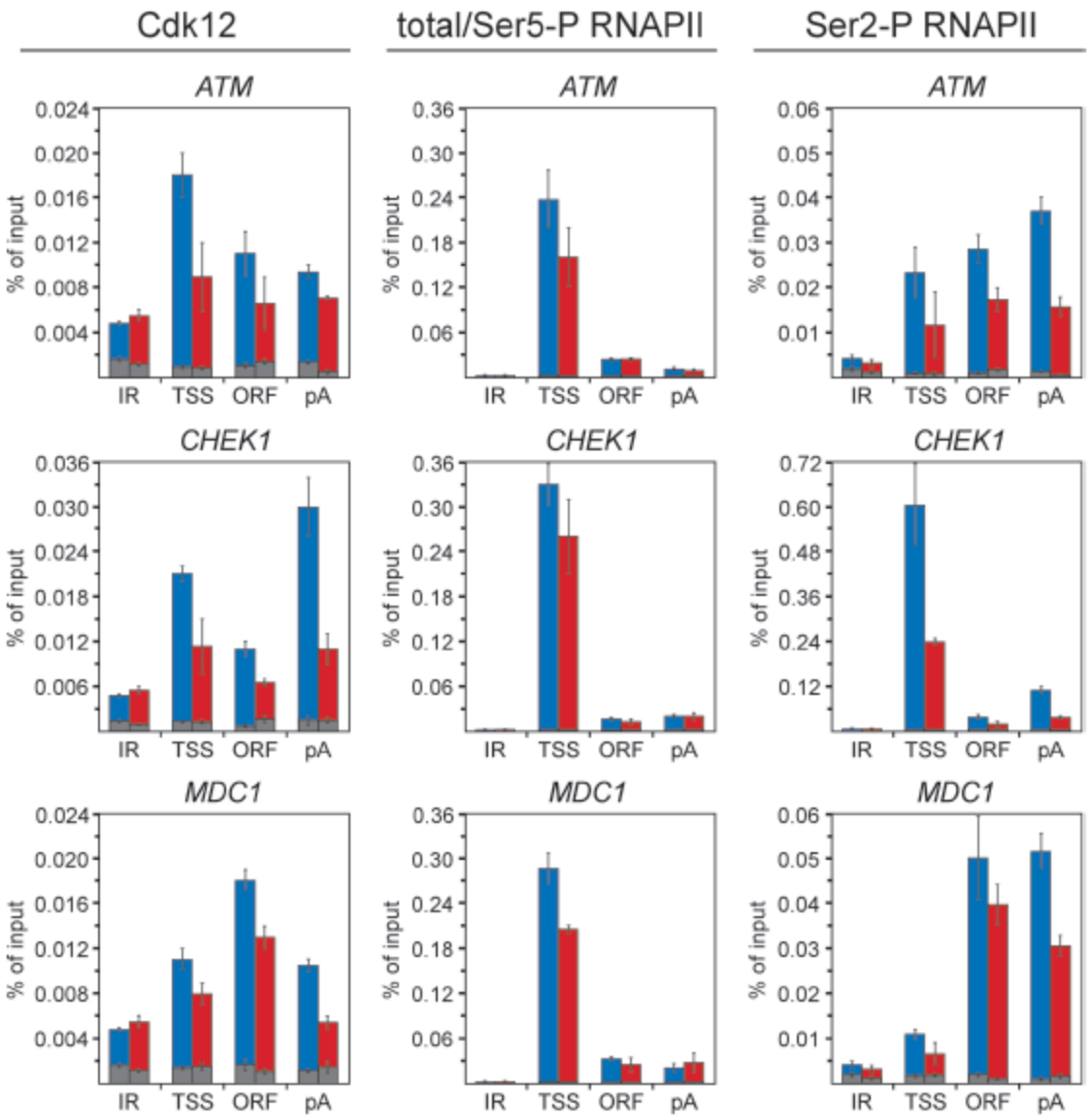
Ekumi, Paculova et al, NAR 2015

Depletion of Cdk12 results in downregulation of HR genes in cell lines



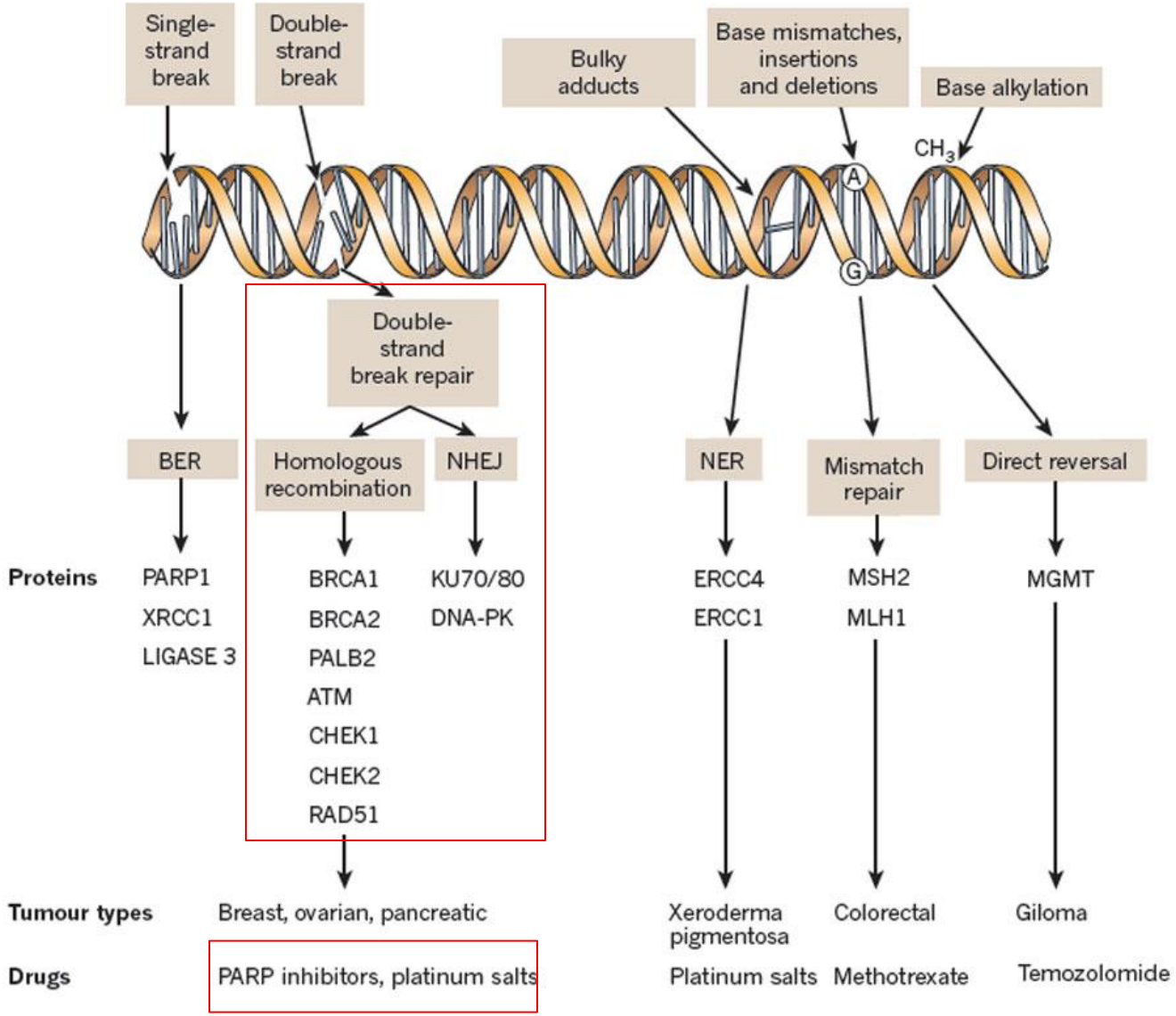
Ekumi, Paculova et al, NAR 2015

Cdk12 is recruited to the DDR genes and regulates Ser2 phosphorylation of the CTD of the RNAPII



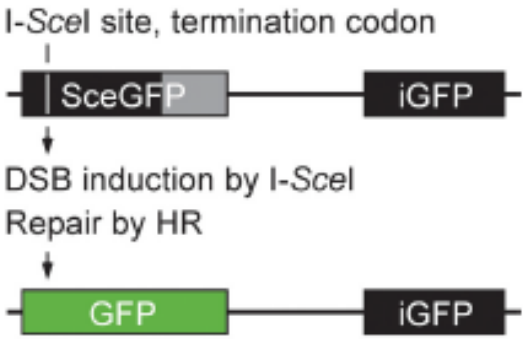
Ekumi, Paculova et al, NAR 2015

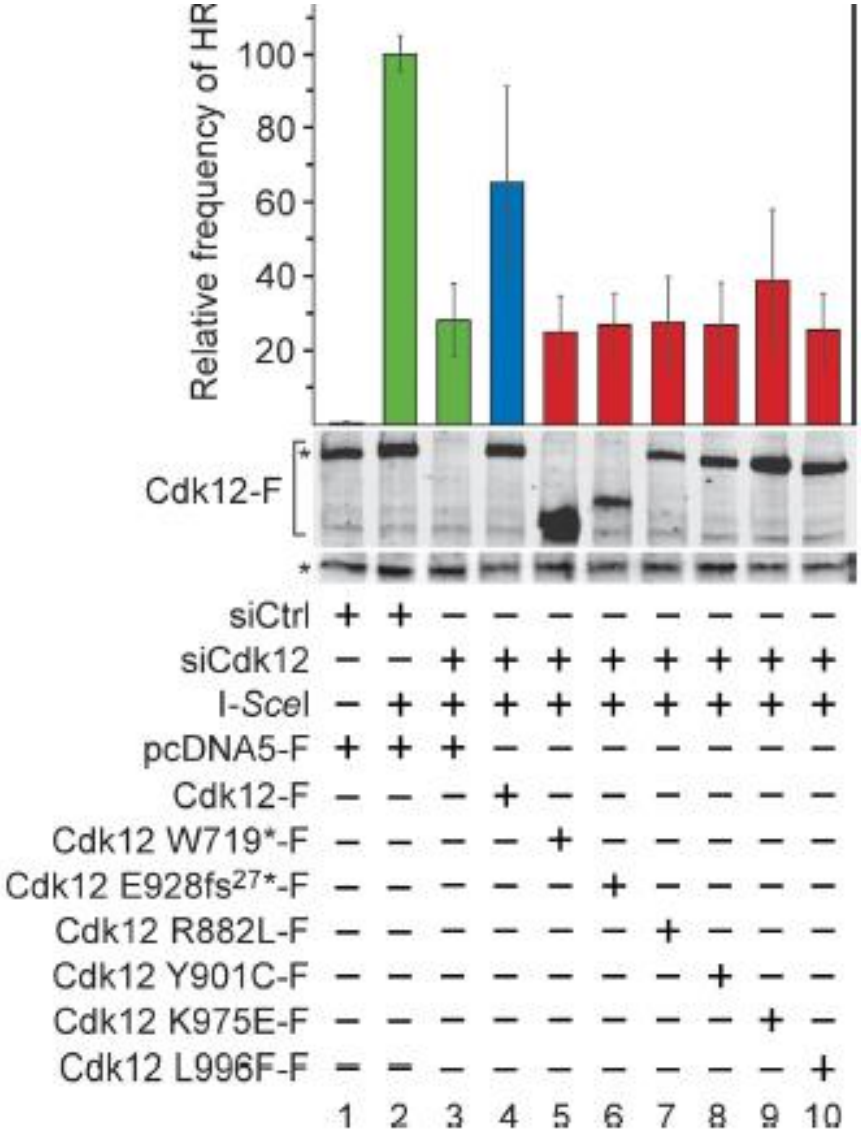
Double-strand breaks are repaired by HR or by non-homologous end-joining (NHEJ)



Lord and Ashworth, Nature 2012

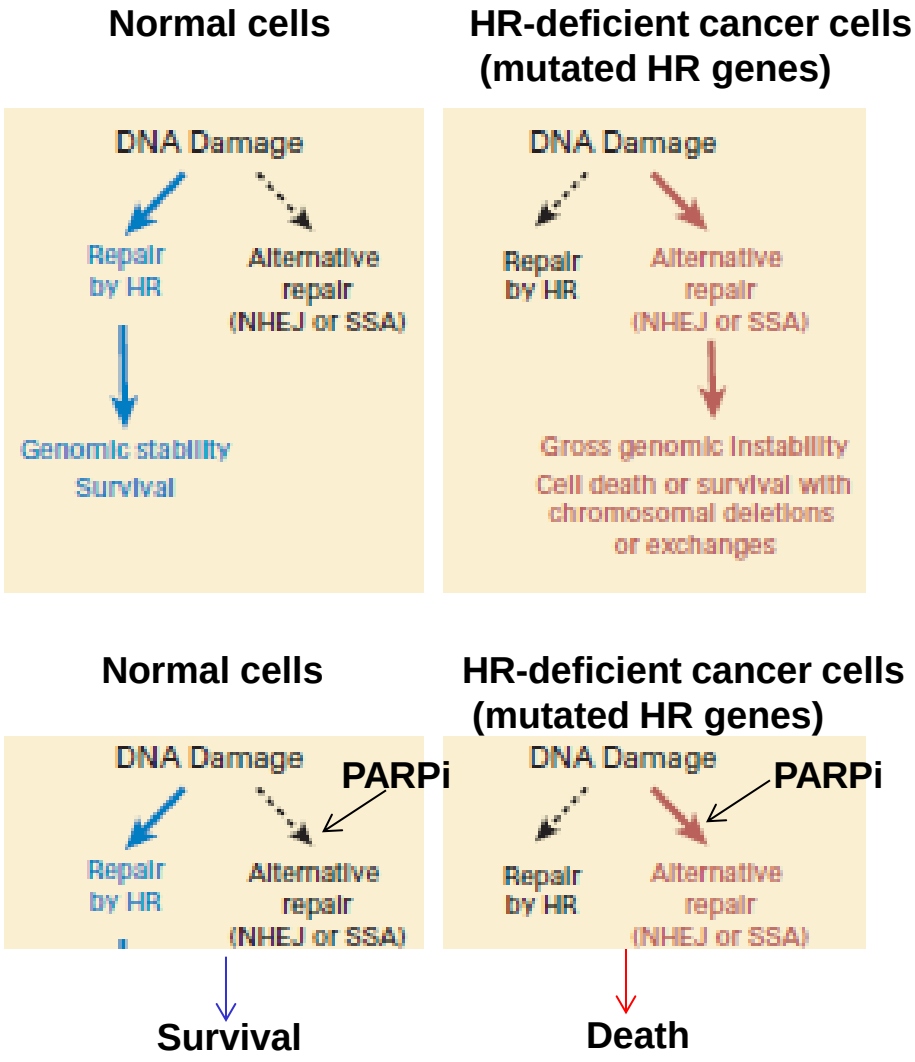
Cdk12 lesions disable the frequency of the repair of double-strand breaks in DNA by HR





Ekumi, Paculova et al, NAR 2015

PARP inhibitors selectively kill HR-deficient cancer cells by inhibiting alternative NHEJ pathway



Adapted from Ashworth, JCI, 2008

Depletion of Cdk12 sensitizes ovarian cancer cells to PARP inhibitors

Cancer Research

AACR

Genome-wide Profiling of Genetic Synthetic Lethality Identifies CDK12 as a Novel Determinant of PARP1/2 Inhibitor Sensitivity

Ilirjana Bajrami, Jessica R. Frankum, Asha Konde, et al.

Cancer Res 2014;74:287-297. Published OnlineFirst November 15, 2013.

Journal of Pathology
J Pathol 2014; **232:** 553–565
Published online 5 February 2014 in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/path.4325

ORIGINAL PAPER

Characterization of the genomic features and expressed fusion genes in micropapillary carcinomas of the breast

Rachael Natrajan,[1,†] Paul M Wilkerson,[1,†] Caterina Marchiò,[2] Salvatore Piscuoglio,[3] Charlotte KY Ng,[3] Patty Wai,[1] Maryou B Lambros,[1] Eleftherios P Samartzis,[4] Konstantin J Dedes,[4] Jessica Frankum,[1] Ilirjana Bajrami,[1] Alicja Kopec,[1] Alan Mackay,[1] Roger A'hern,[5] Kerry Fenwick,[1] Iwanka Kozarewa,[1] Jarle Hakas,[1] Costas Mitsopoulos,[1] David Hardisson,[6] Christopher J Lord,[1] Chandan Kumar-Sinha,[7] Alan Ashworth,[1] Britta Weigelt,[3] Anna Sapino,[2] Arul M Chinnaiyan,[7] Christopher A Maher[8] and Jorge S Reis-Filho[3,*]

Cdk12 mutations cause a defect in HR pathway by collective down-regulation of critical HR genes

Individual Mutations in HR-related Genes:

BRCA1/2
FANCI
ATM
ATR
MDC1
Chek1
Rad51D
….

↓

Defect in HR

↓

HGSOC (PARPi responsive)

Farmer et al, Nature, 2005
McCabe et al, Cancer Research, 2006
Morrison et al, EMBO J, 2007
The Cancer Genome Atlas, Nature, 2011
Lord and Ashworth, Nature, 2012

Individual Mutations in: *Cdk12*

↓

Common Down-regulation of HR-related Genes:

FANCI
ATM
ATR
MDC1
Chek1
Rad51D
(BRCA1)

↓

Defect in HR

↓

HGSOC (PARPi responsive)

Blazek et al, G&D, 2011
Blazek, Cell Cycle, 2012
Bajrami et al, Cancer Research, 2014
Joshi et al, JBC, 2014
Ekumi, Paculova et al, NAR, 2015

Conclusions II

- Most HGSOC *Cdk12* mutations interfere with **Cdk12/CycK complex formation**

- Mutations likely cause structural rearrangements **detrimental to Cdk12 activation**

- **Patient samples** containing the *Cdk12* mutations have **diminished expression of HR genes (ATM, ATR, Rad51D, FANCI)**

- Cells with *Cdk12* mutations **fail to repair DNA double-strand breaks via HR**

- *Cdk12* mutations have a potential to be **markers of PARP inhibitor therapy** in patients with HGSOC

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