

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

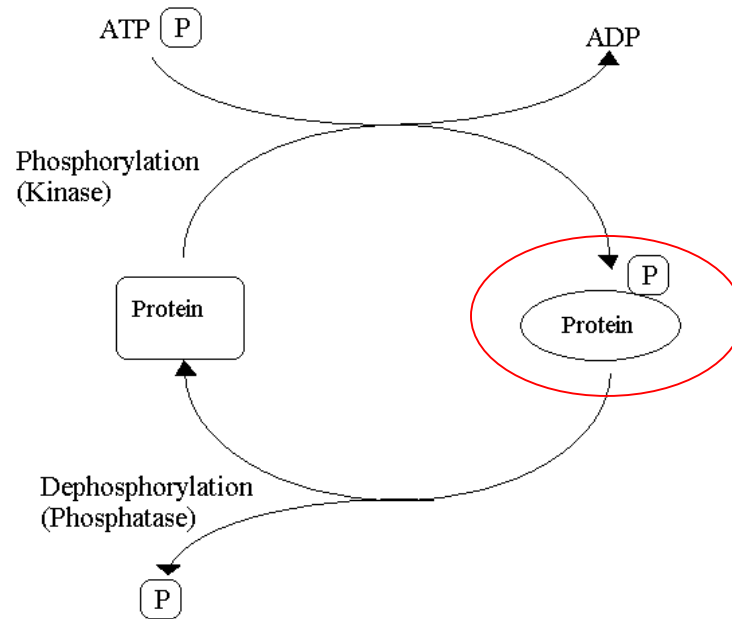
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Molecular Medicine
CEITEC-MU

Summary

- **Kinases**
- **Cyclin-dependent kinases (CDKs)**
- **Cellular functions of cyclin-dependent kinase (CDKs)- in cell cycle
- in transcription**
- **Regulation of cyclin-dependent kinases (CDKs)**
- **Role of cyclin-dependent kinases (CDKs) in human disease (cancer)**
- **Cyclin-dependent kinase (CDKs) as a drug target in treatment of human disease (cancer)**

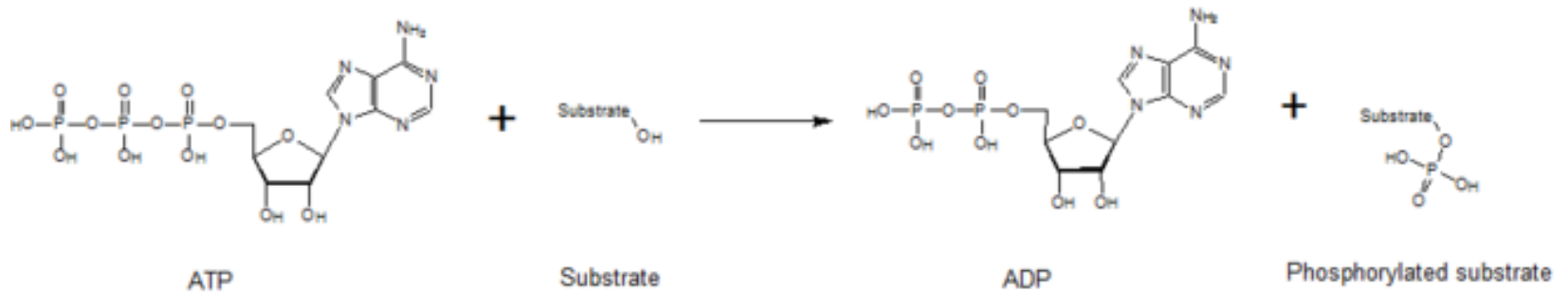
Kinases are enzymes

- Kinases catalyze transfer of a phosphate from high energy molecule such as ATP to a substrate (protein)



- Phosphatases are enzymes catalyze reversible reaction (dephosphorylation)

Kinases are enzymes



- 1954 first time observed phosphorylation of protein casein by Gene Kennedy
- 1956 described reversible phosphorylation/dephosphorylation of proteins by Edmond Fischer and Edwin Krebs (Nobel prize 1992)

Reversible phosphorylation regulates basic cellular processes and its deregulation leads to a disease

Phosphorylation of proteins on four amino acids: serine (Ser)
threonine (Thr)
tyrosine (Tyr)
histidine (His)



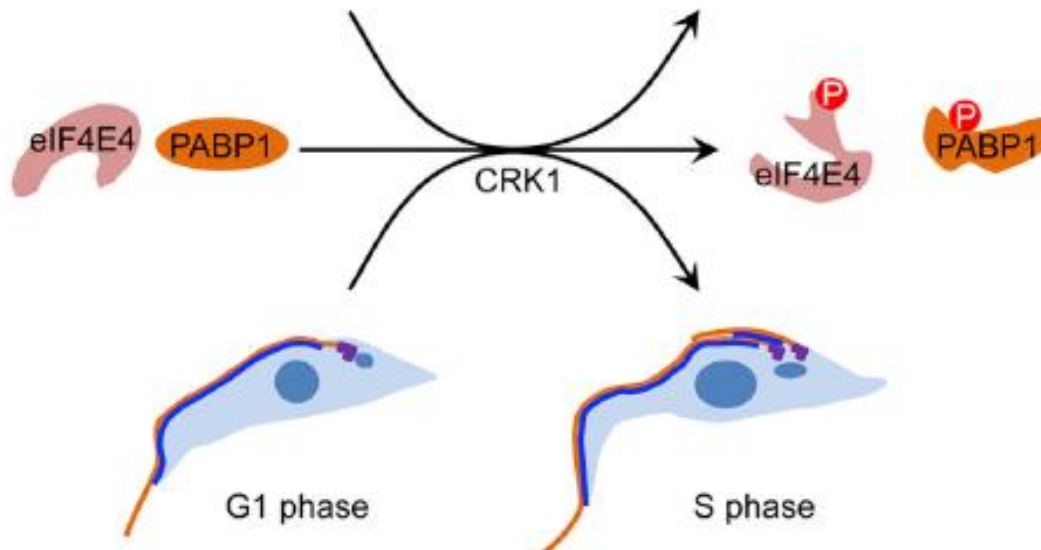
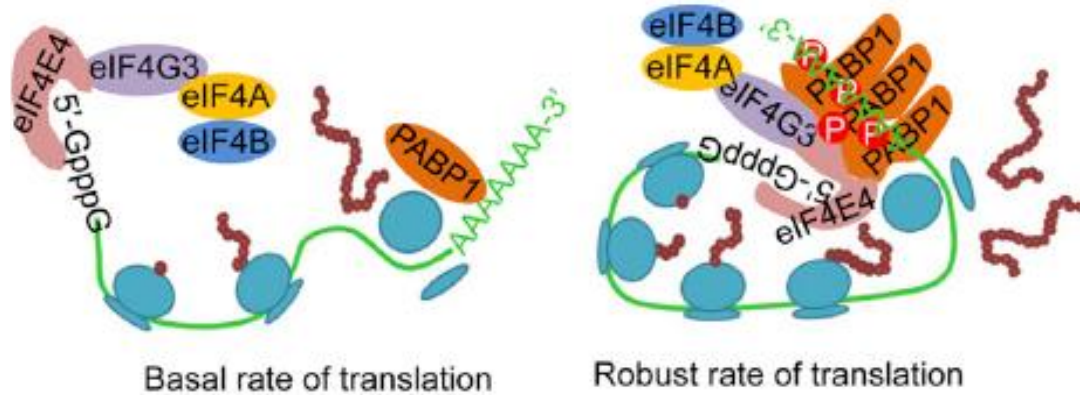
Controls conformation and function of proteins



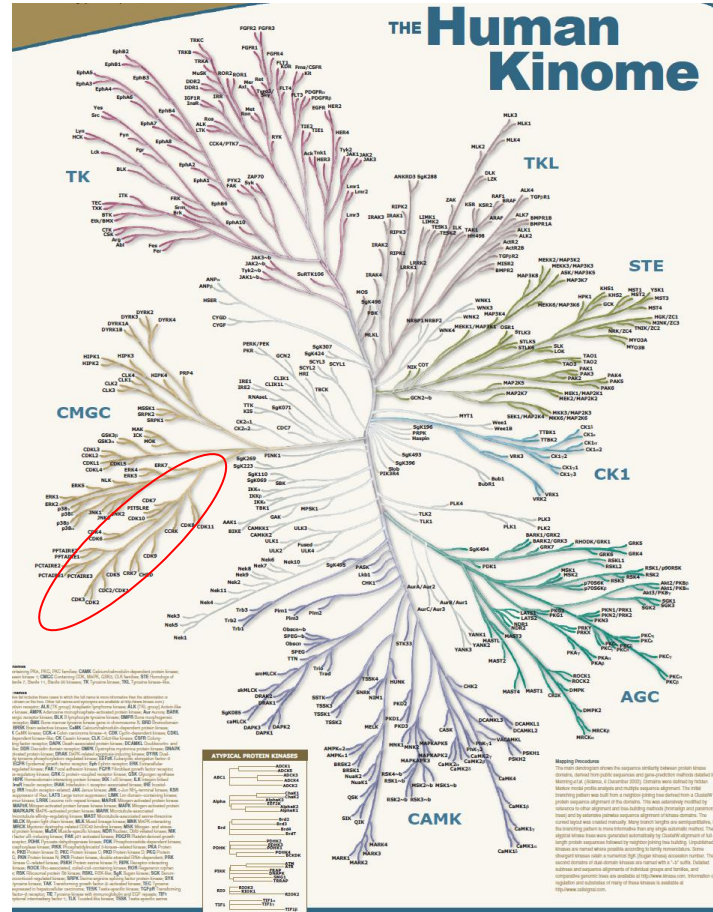
Affects basic cellular processes: protein signaling pathways
cellular metabolism
cellular division
cell cycle
etc.

*(Deregulation of phosphorylation results in disease: cancer
immune diseases
etc.)*

Reversible phosphorylation regulates basic cellular processes and its deregulation leads to a disease



Human kinases

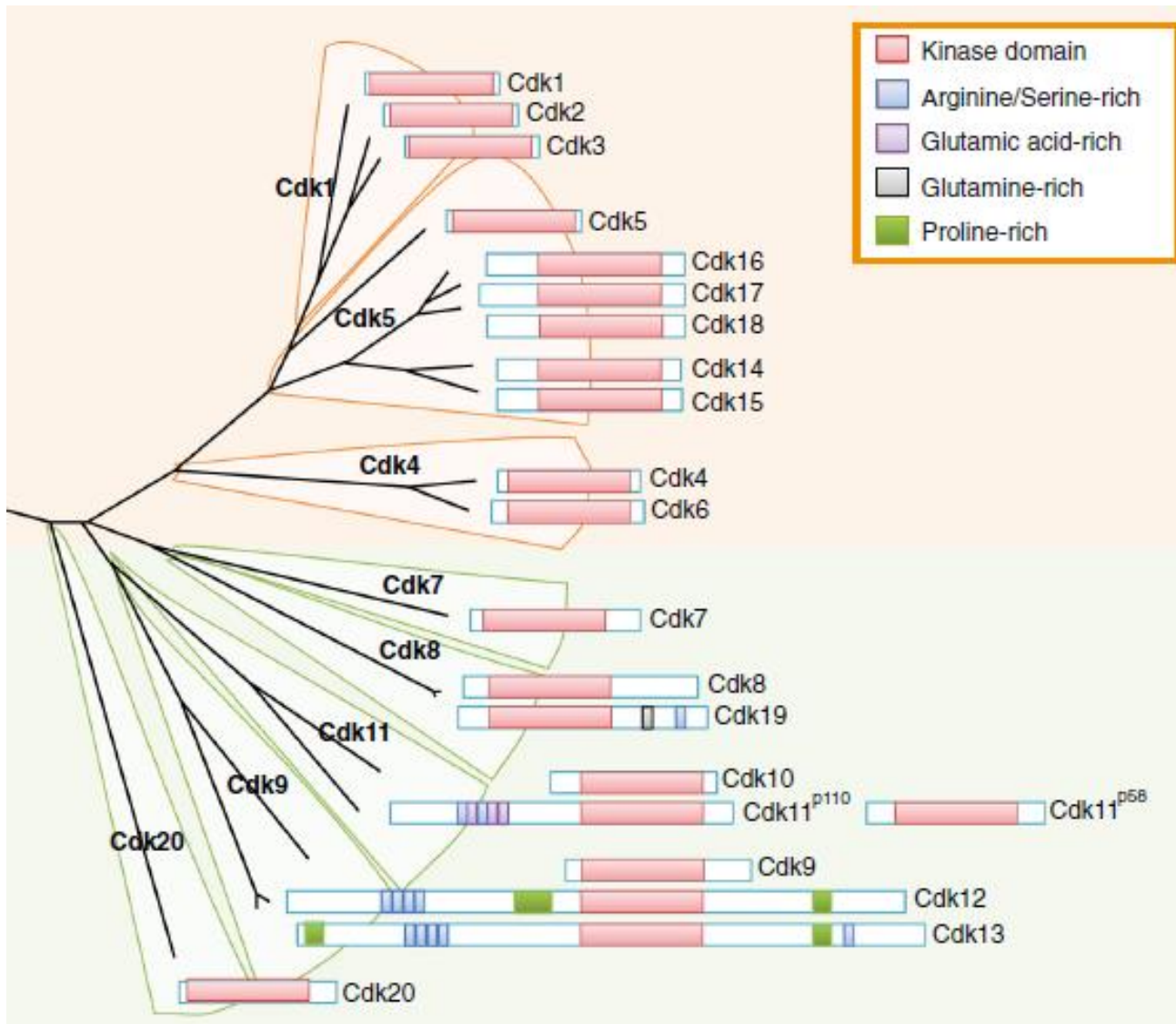


Human kinome consists of 560 kinases divided into 8 groups



= group of **Cyclin-dependent kinases (CDKs)**

Cyclin-dependent kinases (CDKs)



Cyclin-dependent kinases (CDKs)

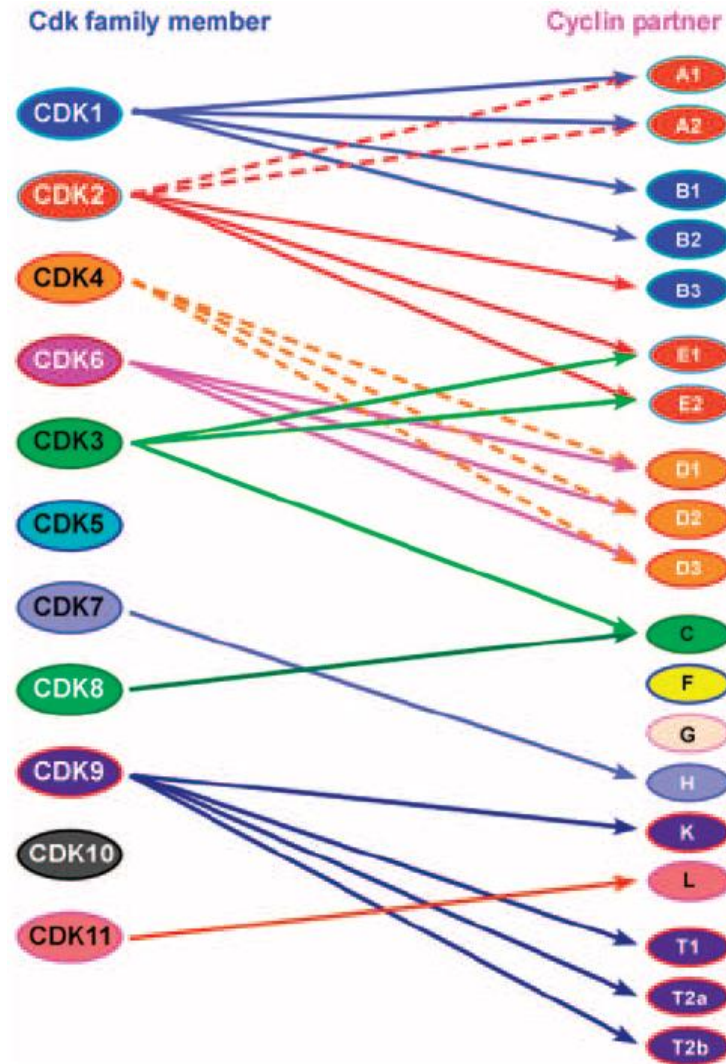


Protein complexes that compose of: 1) CDK (kinase) subunit
2) Cyclin subunit

Both subunits needed for the kinase activity of the complex

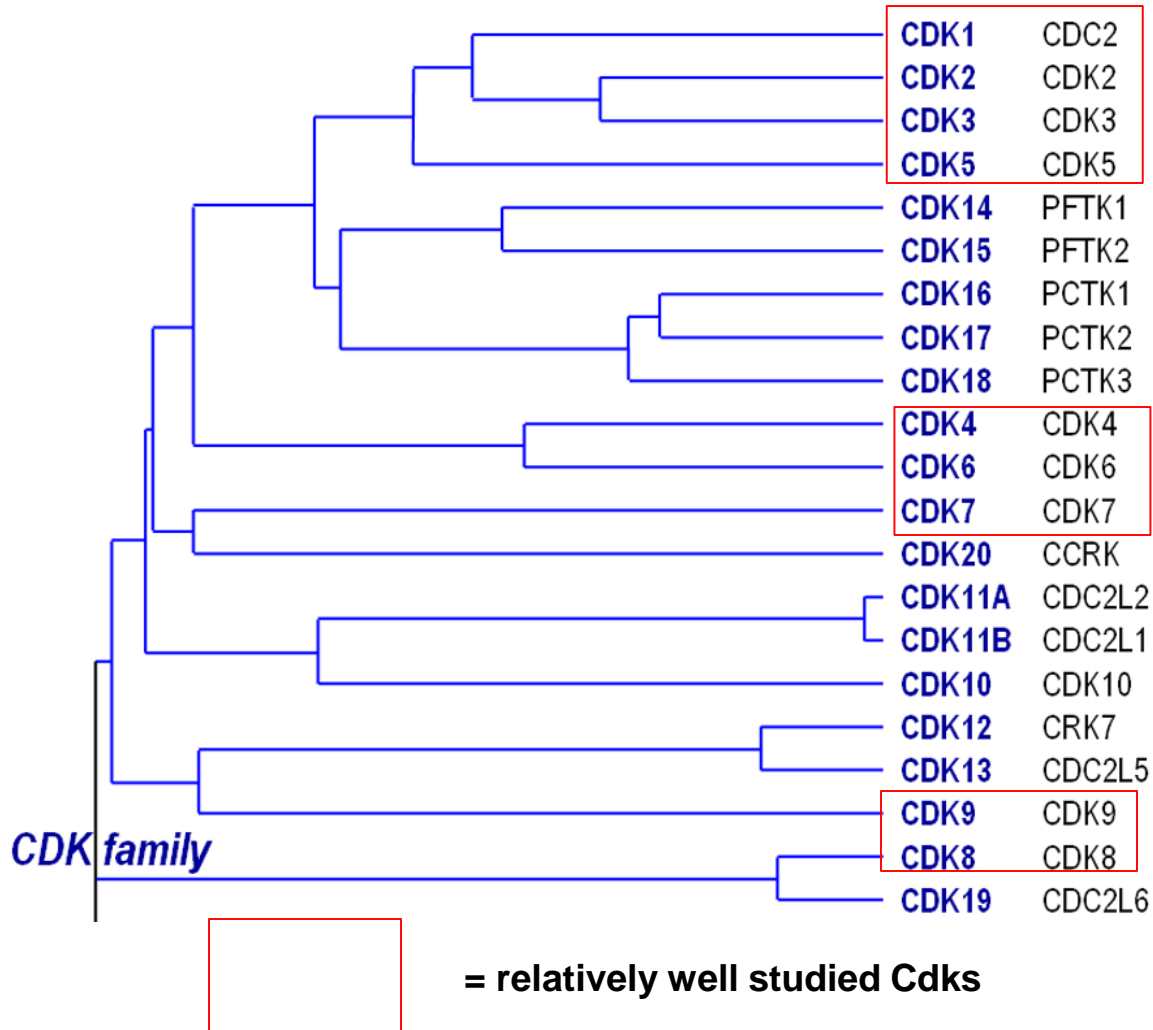
Amino acid sequence preference motif for phosphorylation: S/T-P-X-K/R

CDKs have at least one cyclin partner



Sometimes cyclin subunit regulates specificity towards substrate, sometimes redundant

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

Regulation of cellular functions by CDKs

The CDK complexes regulate various processes in cells

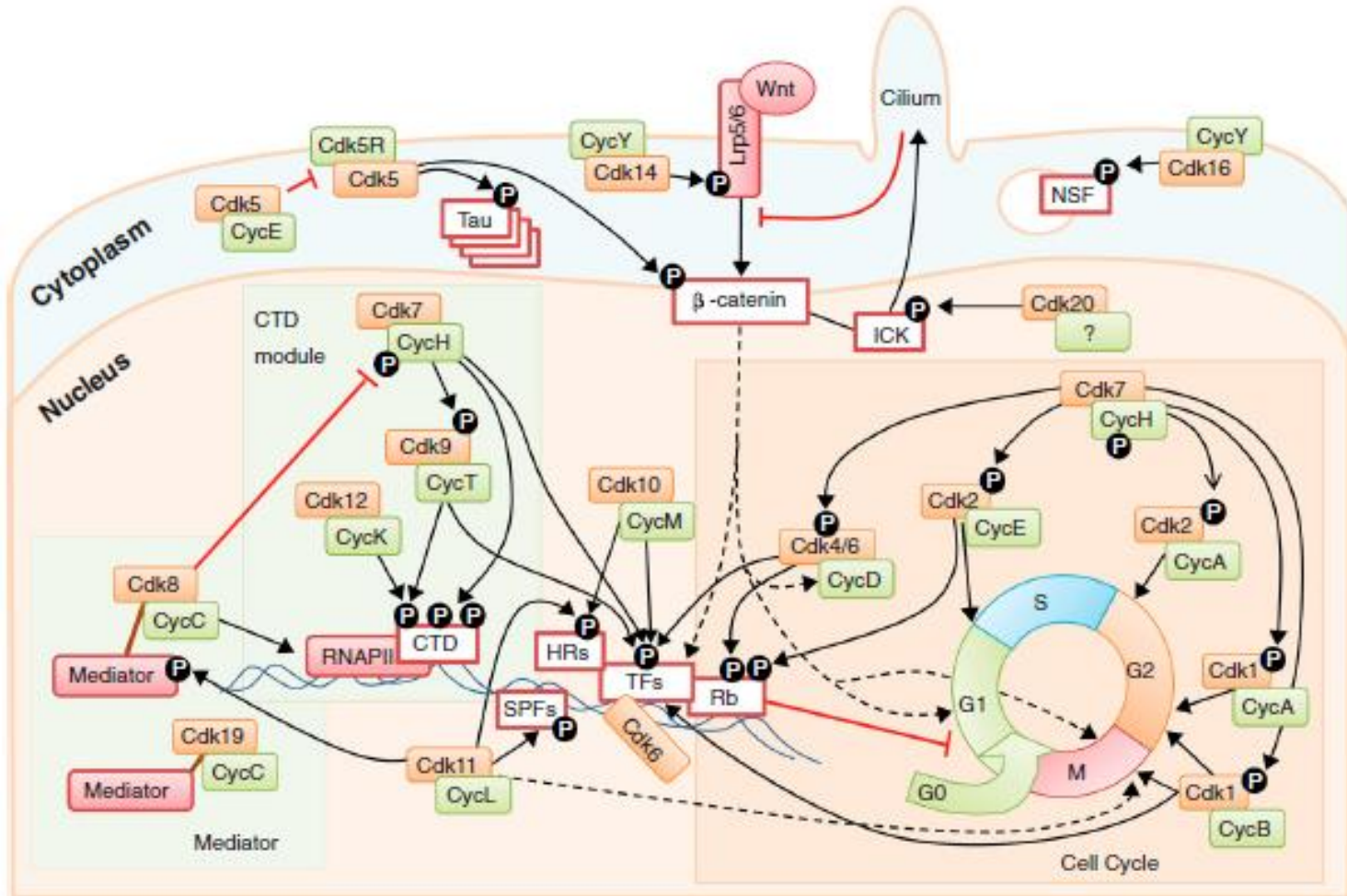
Major functions:

- Regulation of Cell Cycle (CDK1,2,4,6,7)
- Regulation of Transcription (CDK7,8,9,11,12)

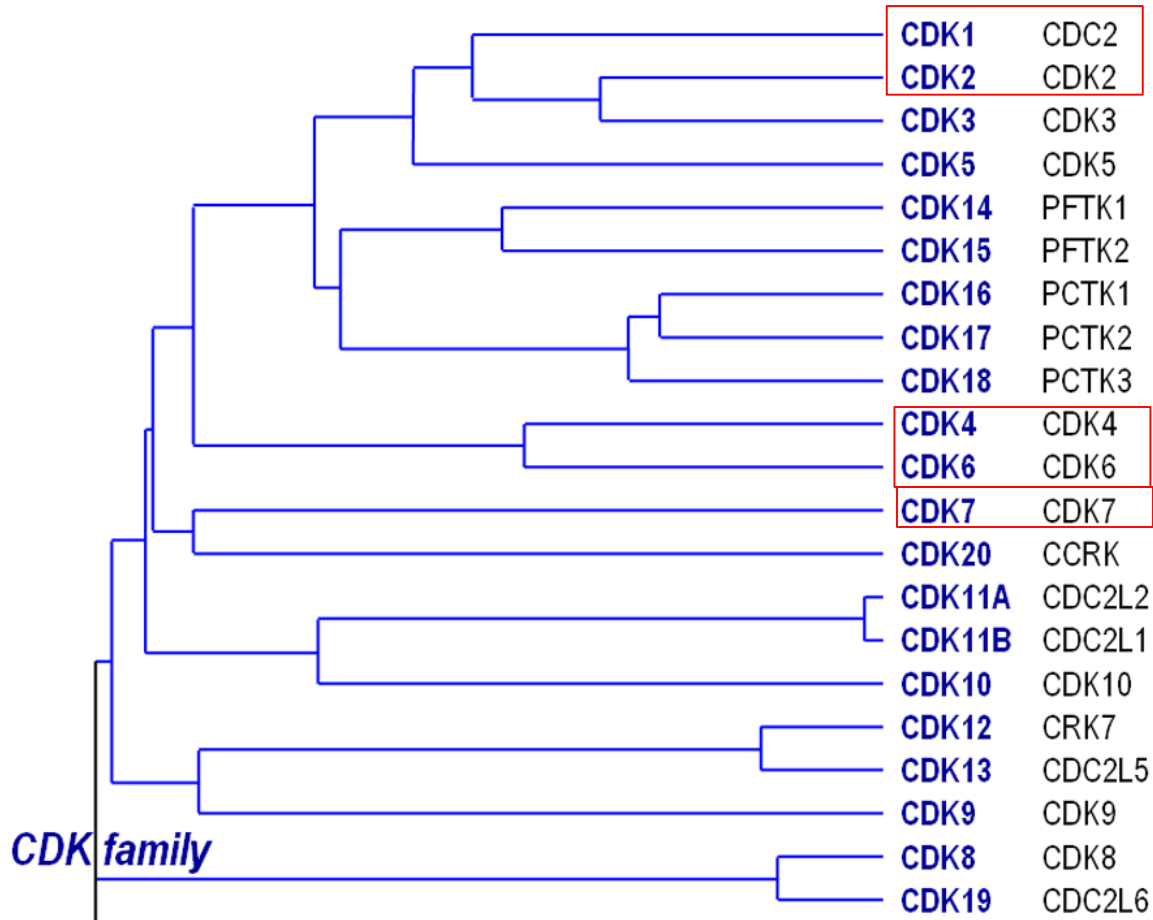
Other functions:

- regulation of pre-mRNA processing (CDK11, CDK9)
- regulation of neuronal cell differentiation (CDK5)
- likely more functions to be discovered

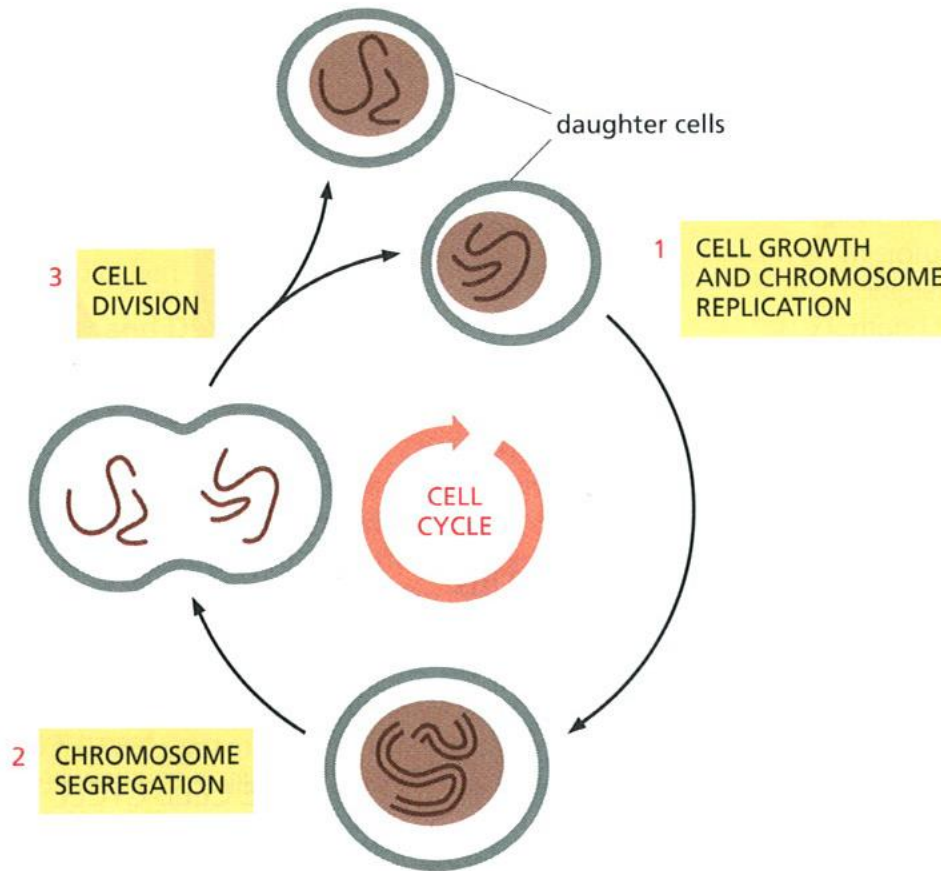
CDK complexes regulate various processes in cells



Regulation of Cell Cycle by CDKs

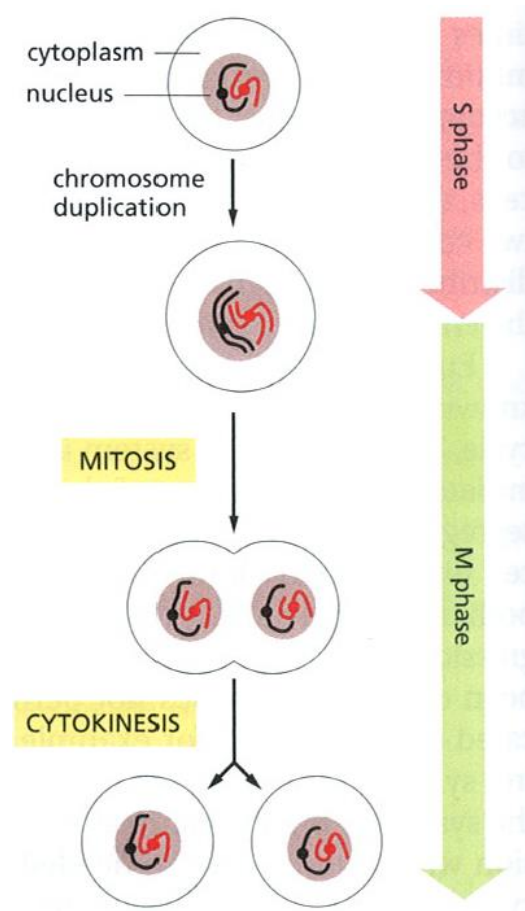


Cell Cycle



Cell cycle leads to production of two genetically identical daughter cells

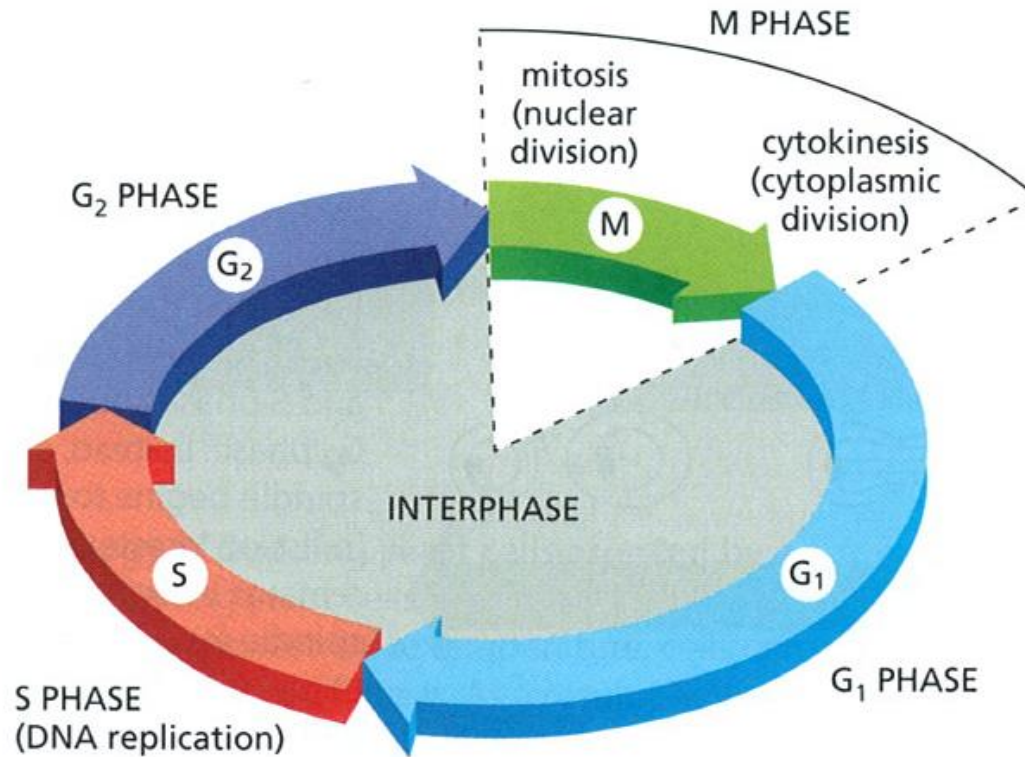
Major events of the cell cycle



S-phase – DNA synthesis-duplication of the chromosomes

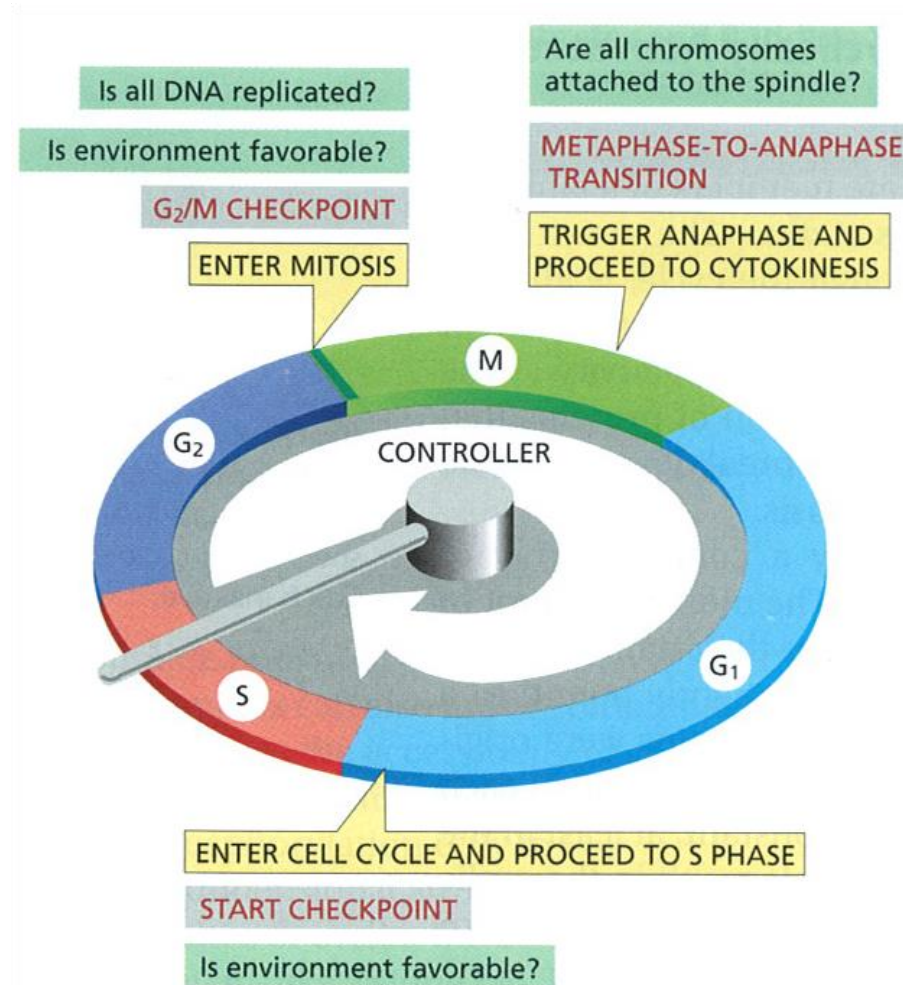
**M-phase – mitosis-pair of chromosomes segregated into the nuclei
– cytokinesis- the cell divides into two identical cells**

The cell cycle has four phases



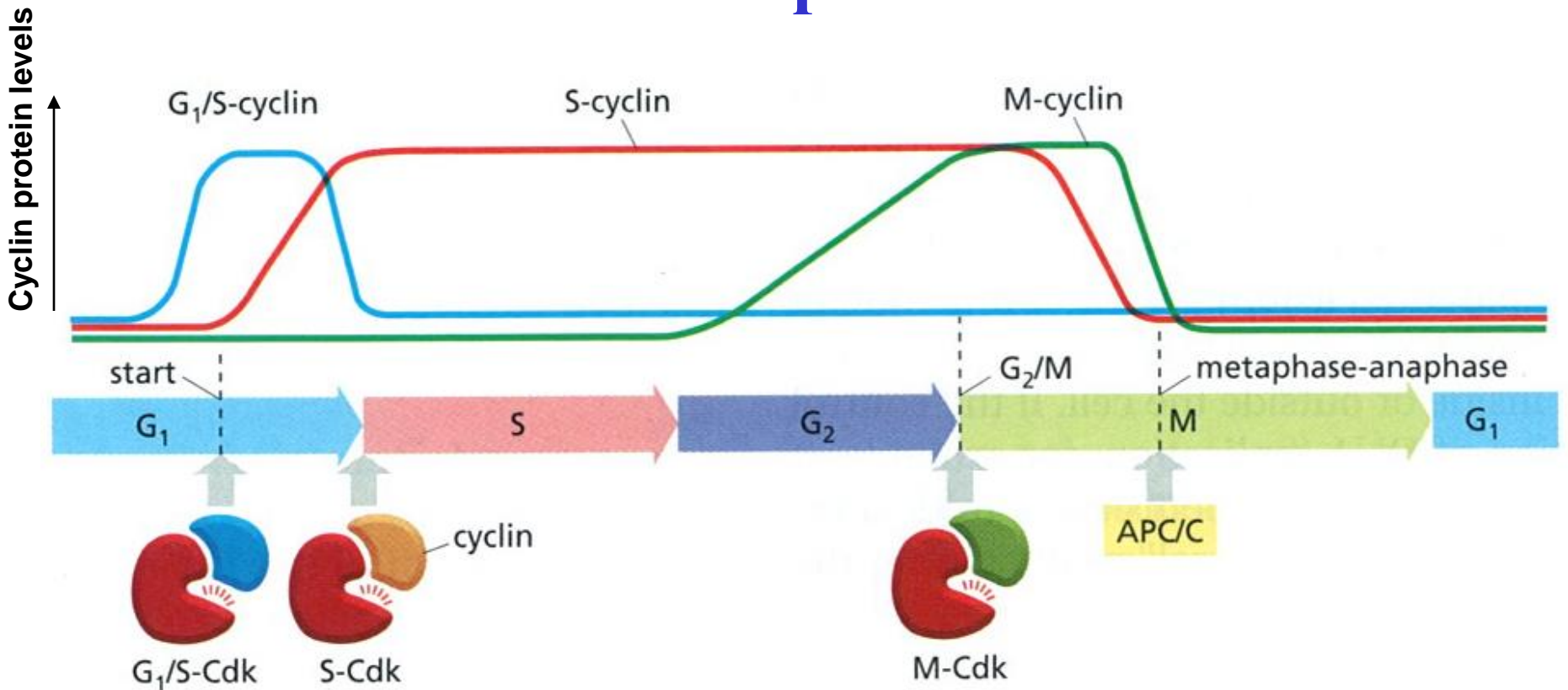
G₁ and G₂ phases-time delay to allow the growth of the cell
-time to monitor external and internal conditions before commitment to onset of S and M phase

The control of the cell cycle: three major “checkpoints”



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

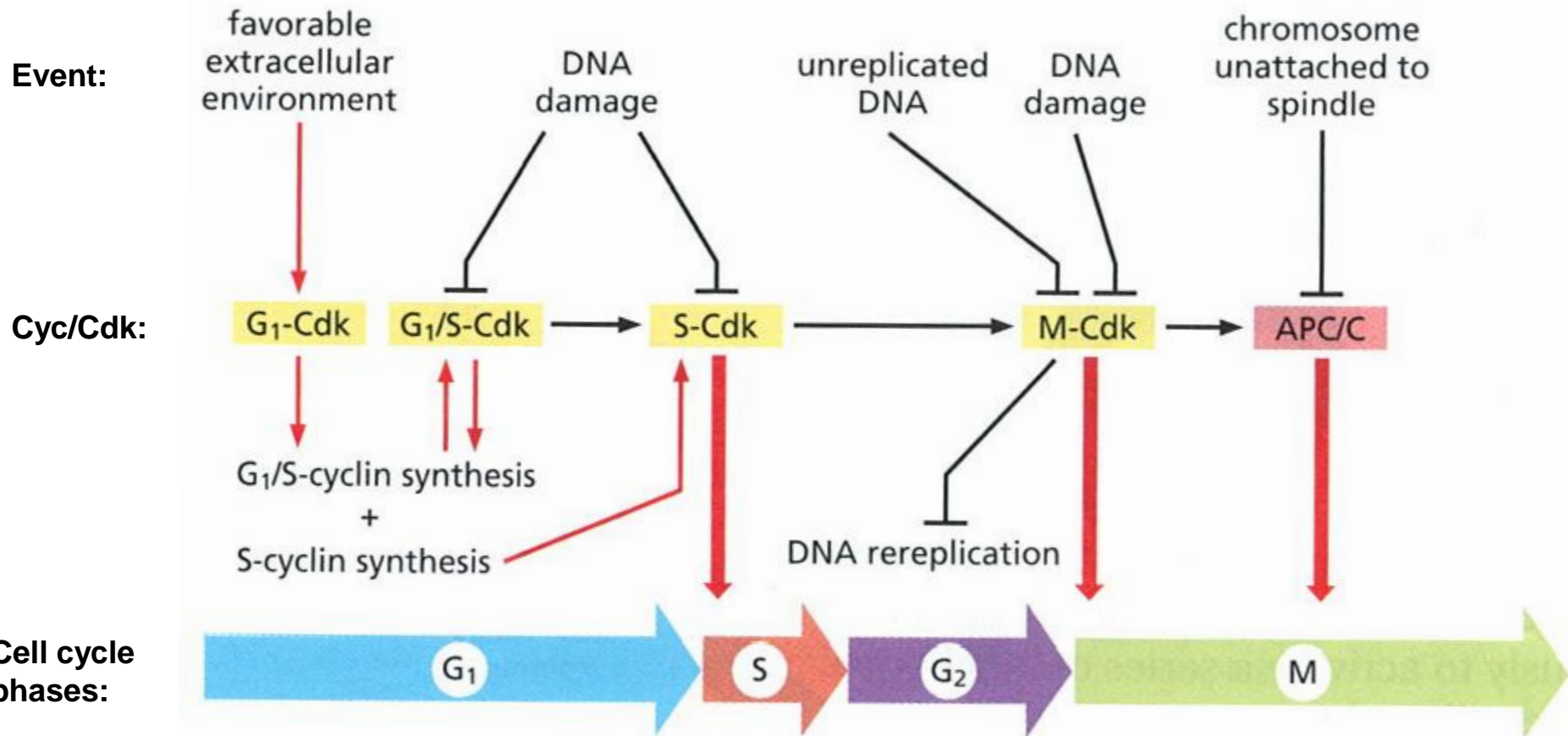
Cell cycle control system depends on cyclically activated CDKs that control three major “checkpoints”



Cyclin protein levels change, CDK protein levels are constant

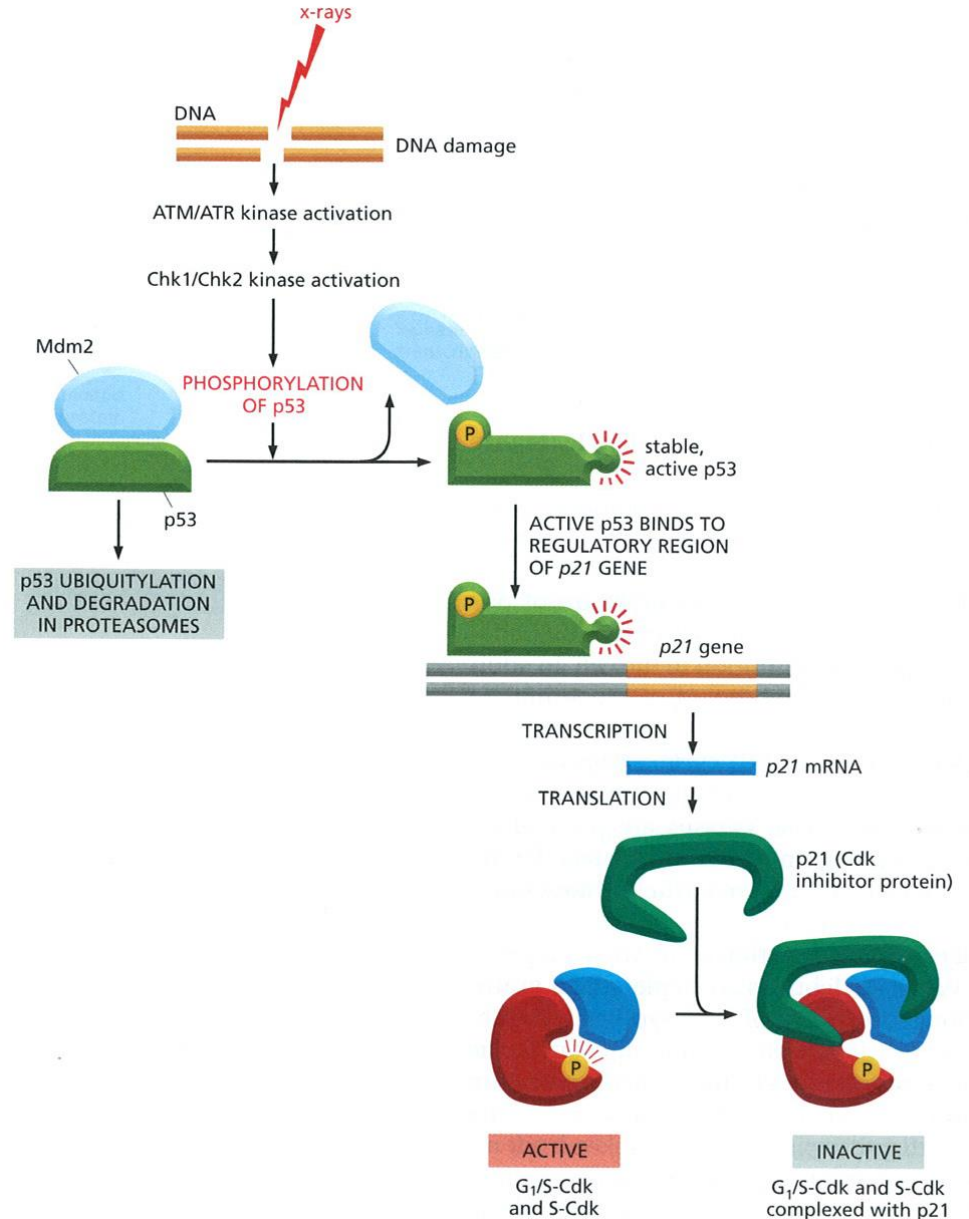
Cyclical changes (expression and degradation) in Cyclin protein levels result in cyclic assembly/disassembly and activation/inhibition of Cyc/CDK complexes; this leads to phosphorylation/dephosphorylation of proteins that initiate and regulate cell cycle events

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

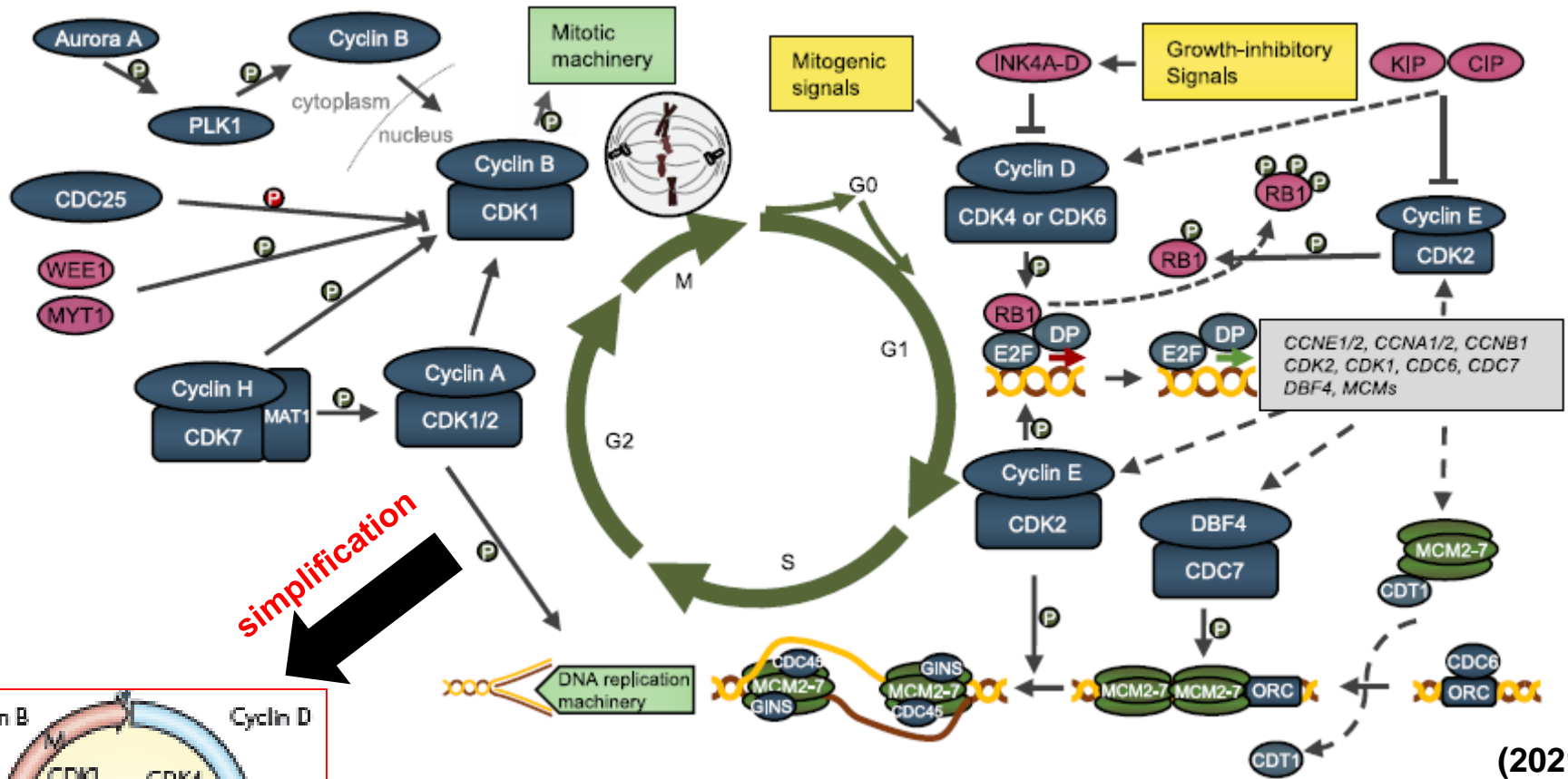


Example: Mechanism of cell cycle arrest in G1 by DNA damage

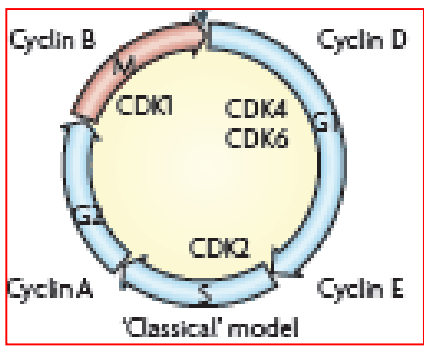
DNA damage causes transcription of p21, Cdk inhibitory protein, that inhibits G1-S- and S-Cdks, arresting the cell cycle in G1 phase



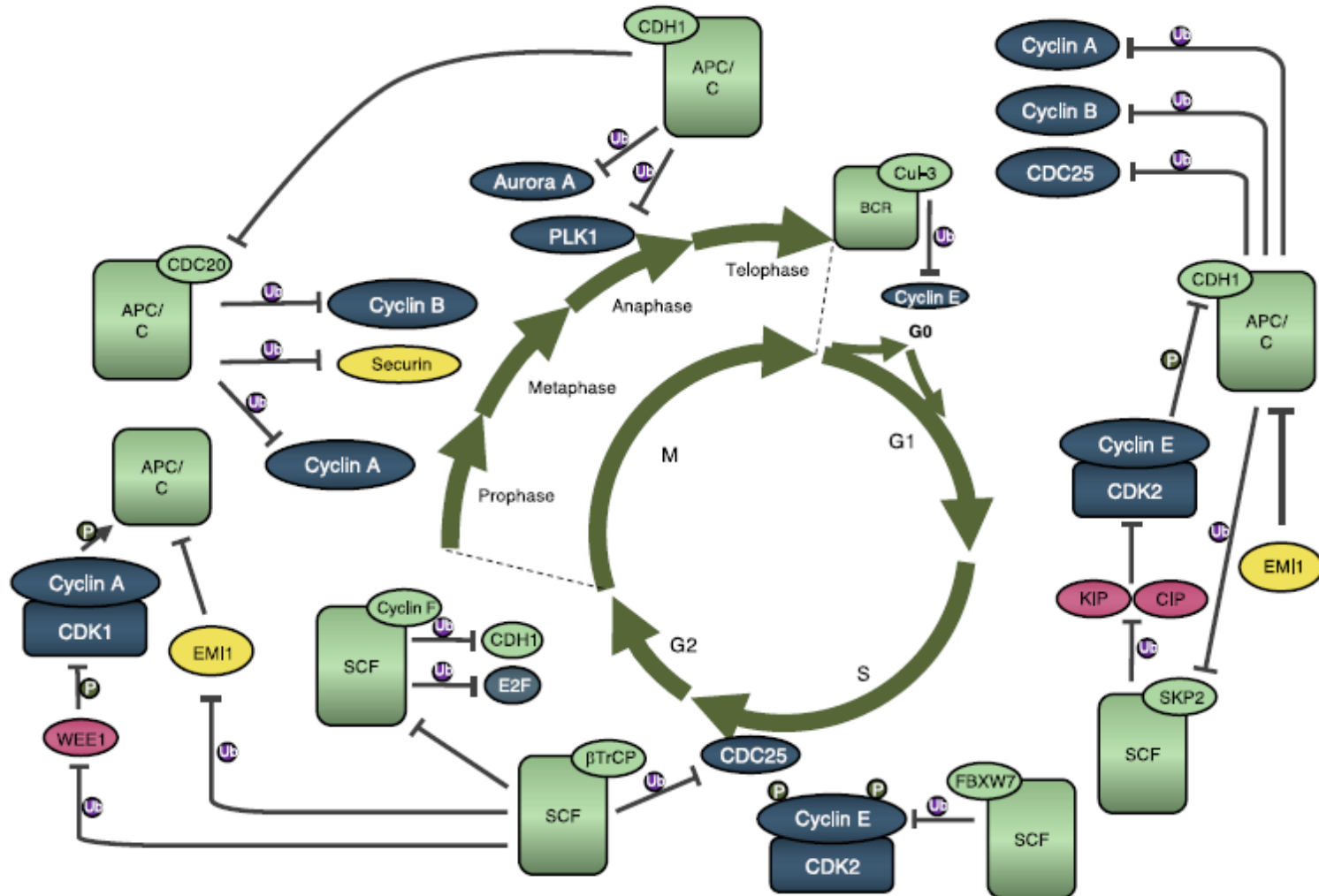
Overview of cyclin/CDK-regulated cell cycle progression



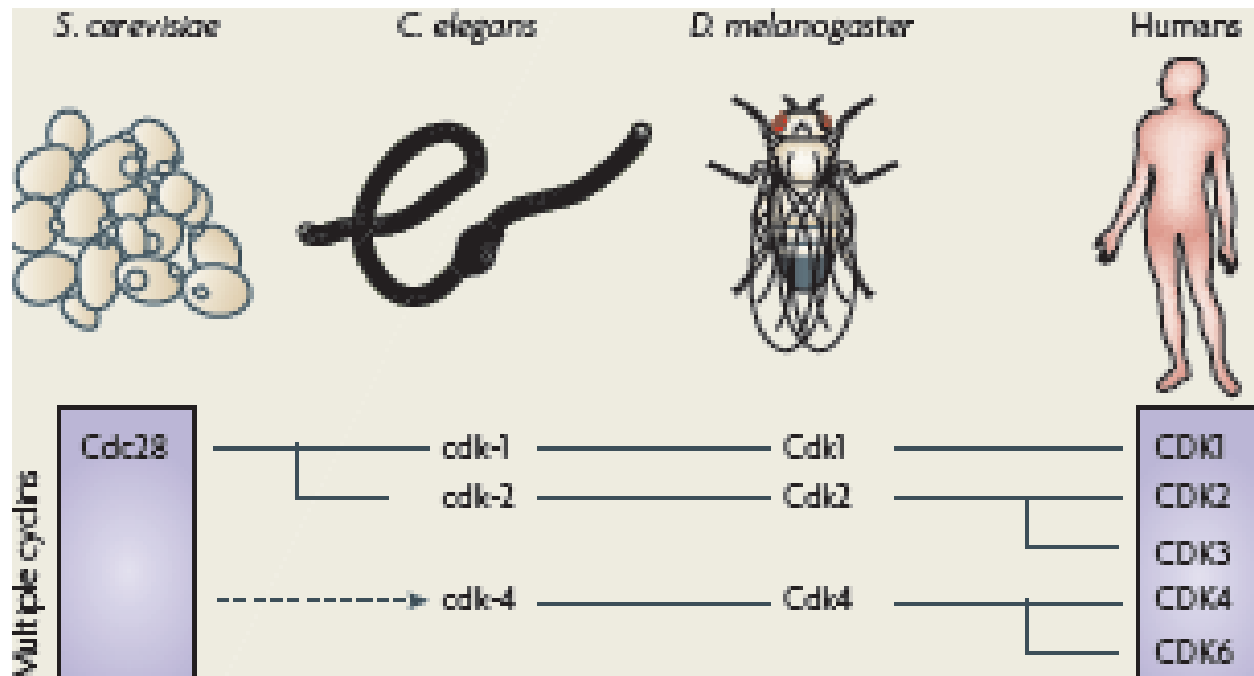
simplification



Overview of regulation of cell cycle machinery (cyclins and other proteins) by proteasomal degradation



Evolution of cell cycle control



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)

Major Cyclins and CDKs in Vertebrates and Yeast

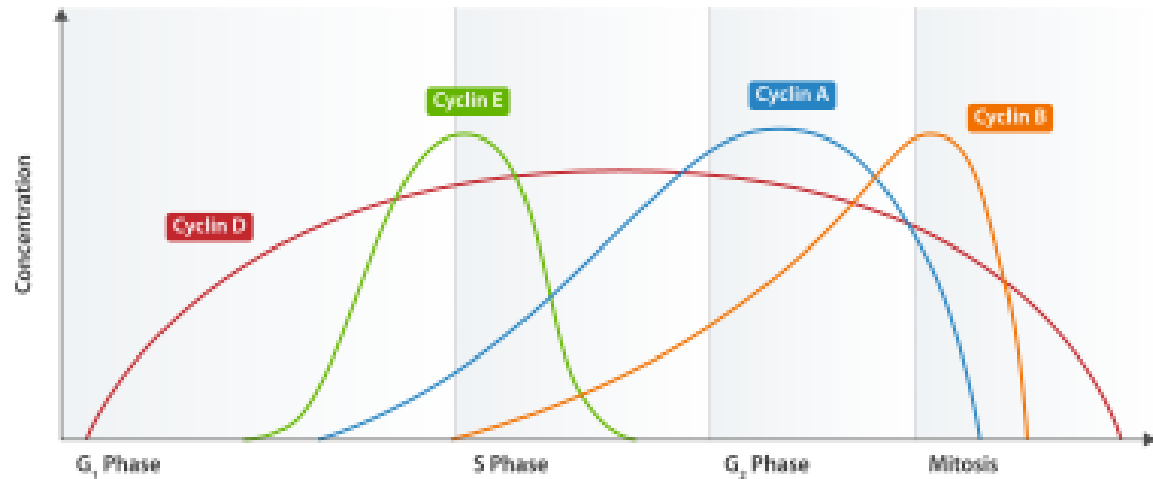
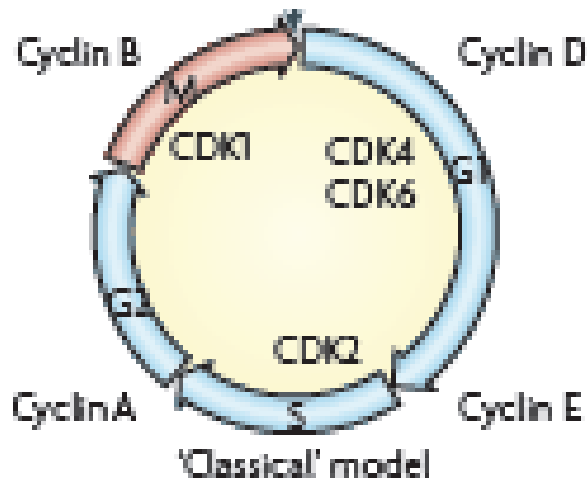
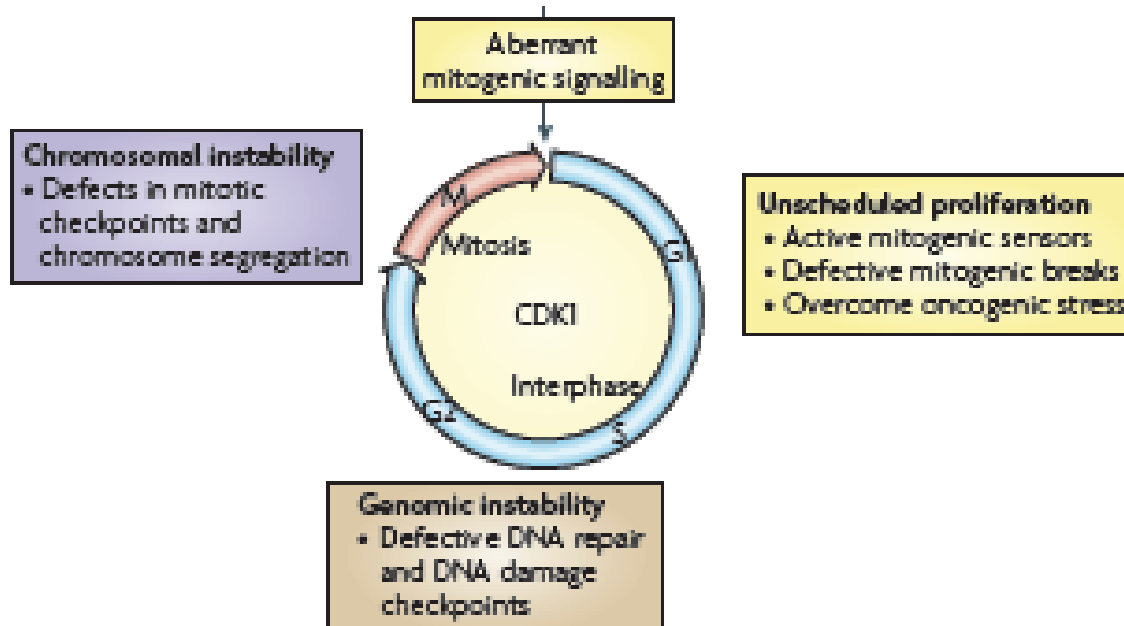


Table 17-1 The Major Cyclins and Cdks of Vertebrates and Budding Yeast

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

Deregulation of cell cycle and cancer



Cells escape from the proper control of the cell cycle during cancer development:

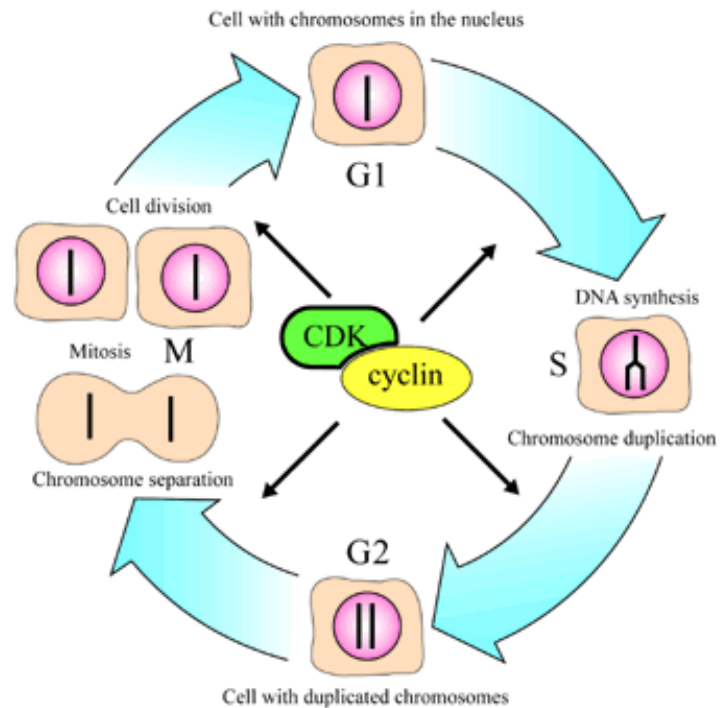
- Increase in expression and activity of CDKs
- Inactivation of inhibitors (regulators) of CDKs



**Consequences: Unscheduled proliferation
Genomic instability
Chromosomal instability**

Nobel prize (2001) for discovery of “key regulators of the cell cycle”

The Cell Cycle



Leland H. Hartwell
Prize share: 1/3

Concept of
“Checkpoint”
(early 1970s)



Tim Hunt
Prize share: 1/3

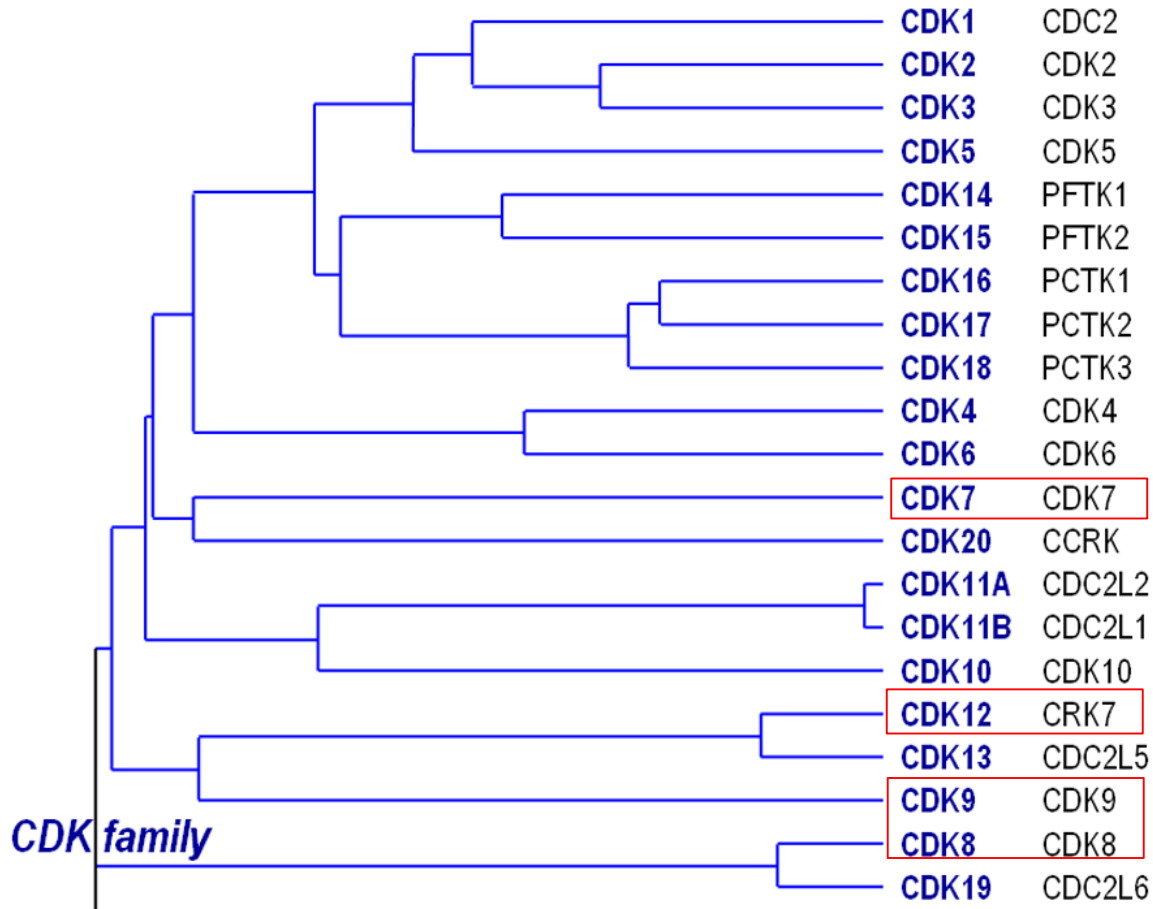
Discovery of cyclin
(early 1980s)



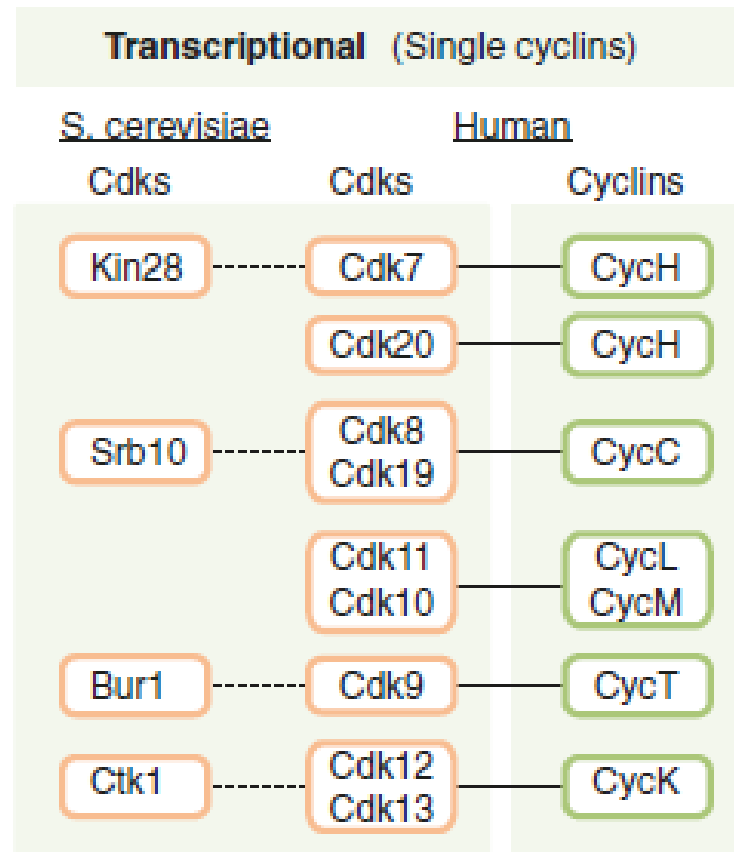
Sir Paul M. Nurse
Prize share: 1/3

Discovery of CDK
(mid 1970s)

Regulation of transcription by CDKs

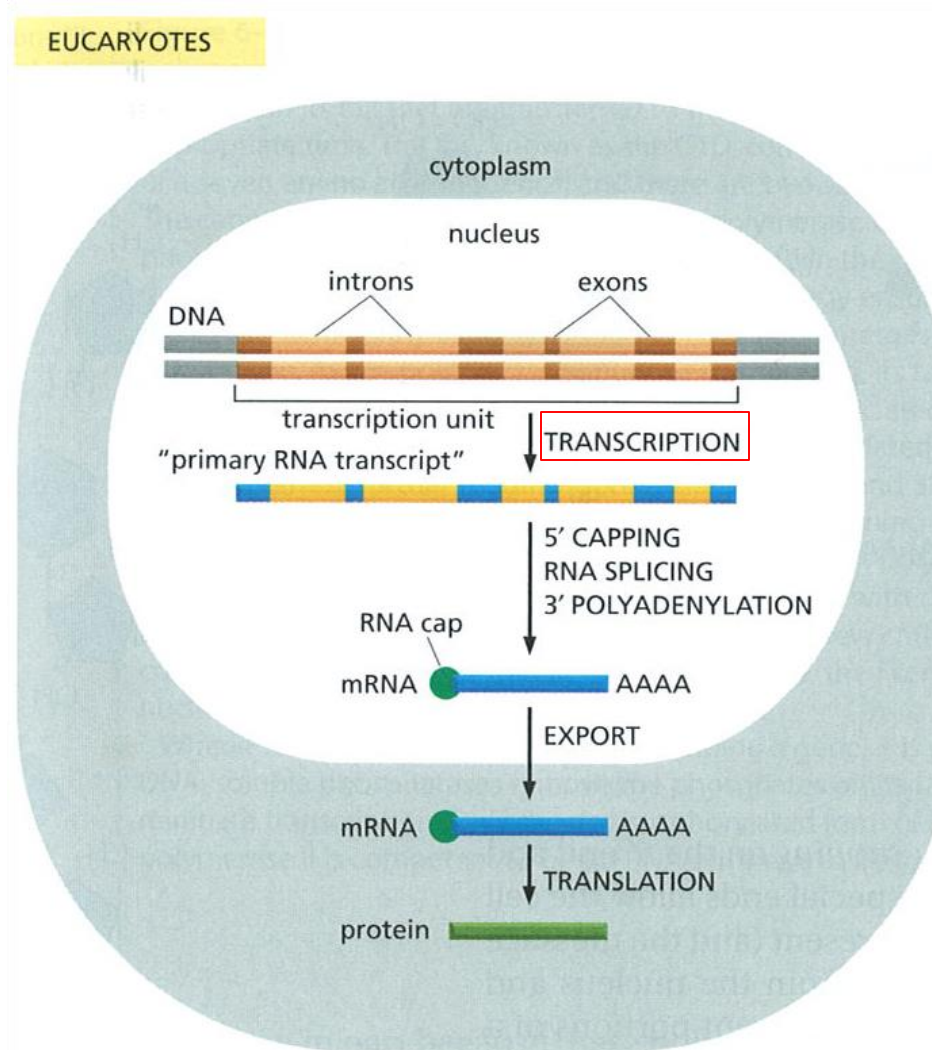


Transcriptional Cyc/CDK complexes



More CDKs in human versus in yeast likely reflects higher complexity of human genomes; i.e. human genes are longer, have introns etc \longrightarrow more regulation needed

Transcription (Gene expression)



Transcription: synthesis of RNA from DNA template (gene)

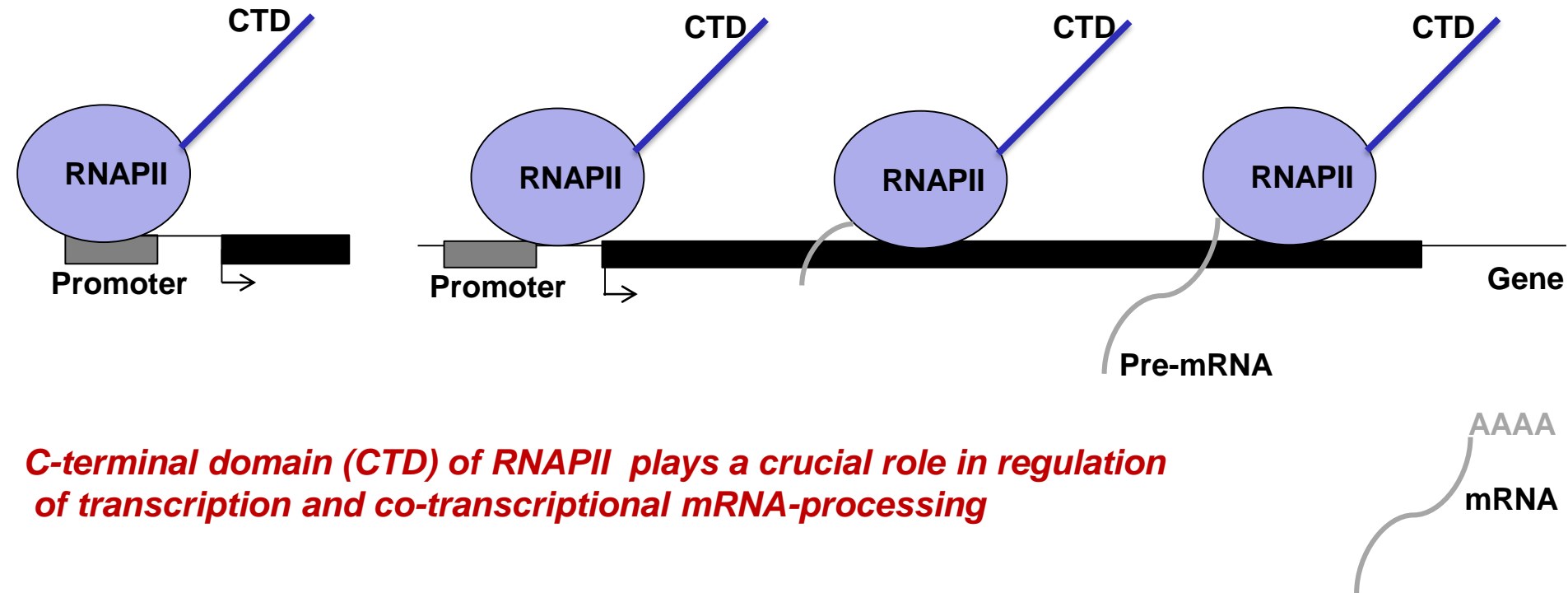
Transcription of protein-coding genes by RNA polymerase II (RNAPII)

Pre-initiating RNAPII

Initiation

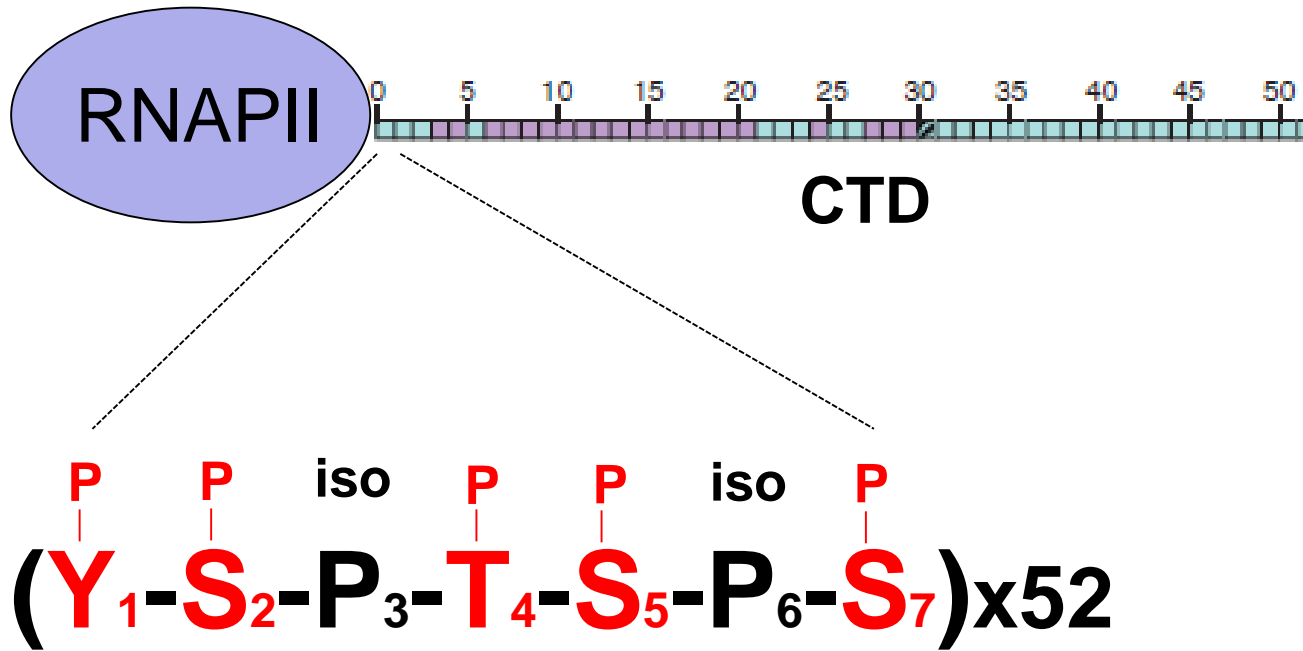
Elongation

Termination



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

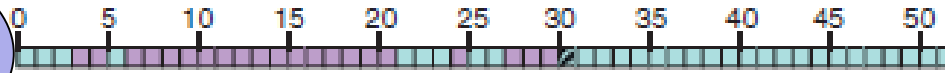
**CTD consists of 52 repeats of heptapeptide YSPTSPS
in which individual amino acids get phosphorylated
to form a “CTD code”**



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

Human “CTD code”

RNAPII



Key: Consensus
 Non-consensus
 Site-specific modification (R1810)

Phosphorylation state

16 combinations

YSPTSPS	none
YSPTSPS	S2
YSPTSPS	T4
YSPTSPS	S5
YSPTSPS	S7
YSPTSPS	S2, T4
YSPTSPS	S2, S5
YSPTSPS	S2, S7
YSPTSPS	T4, S5
YSPTSPS	T4, S7
YSPTSPS	S5, S7
YSPTSPS	S2, T4, S5
YSPTSPS	S2, T4, S7
YSPTSPS	S2, S5, S7
YSPTSPS	T4, S5, S7
YSPTSPS	S2, T4, S5, S7

Proline isomerization state

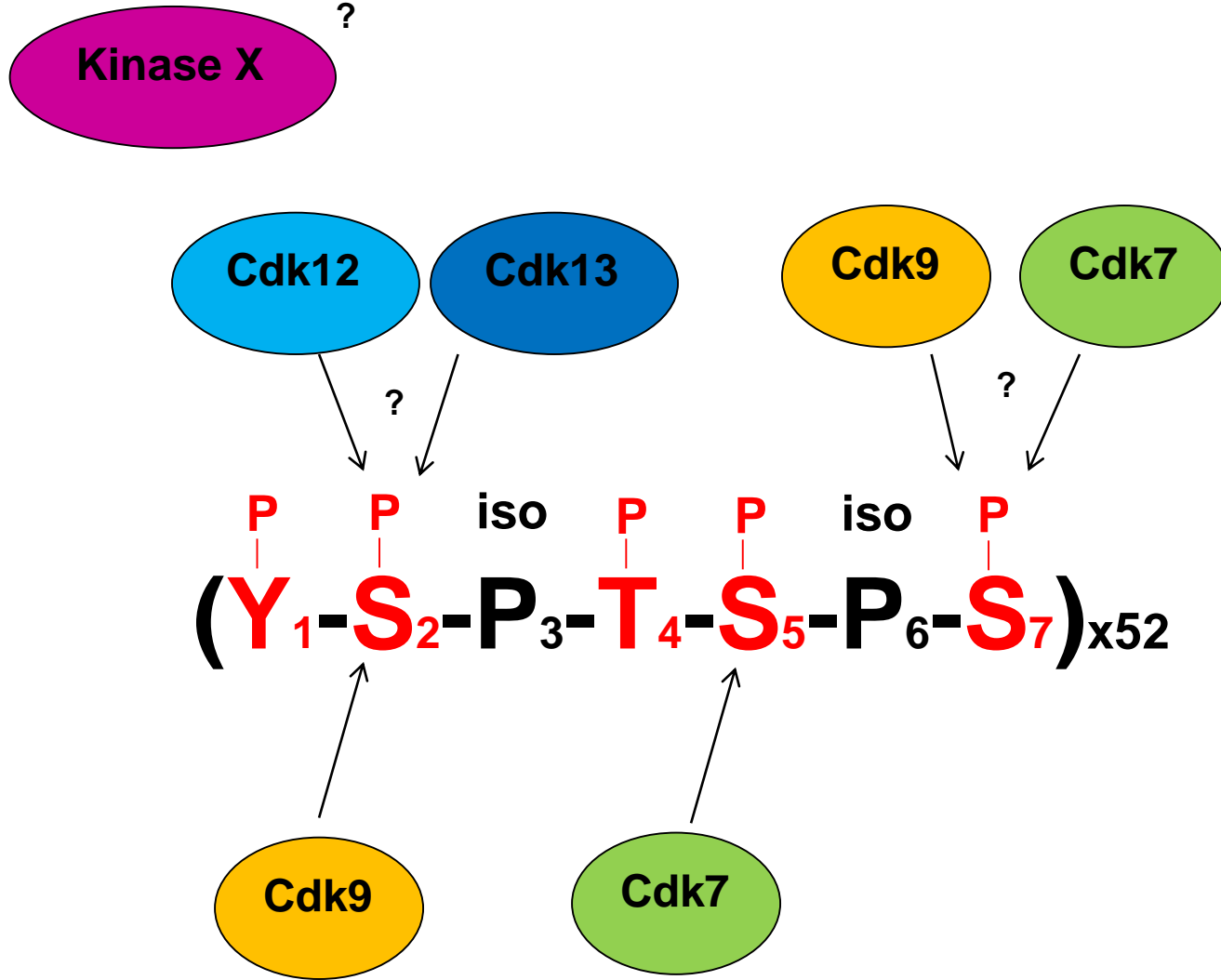
4 combinations

YSPTSPS	<i>cis, cis</i>
YSPTSPS	<i>cis, trans</i>
YSPTSPS	<i>trans, cis</i>
YSPTSPS	<i>trans, trans</i>

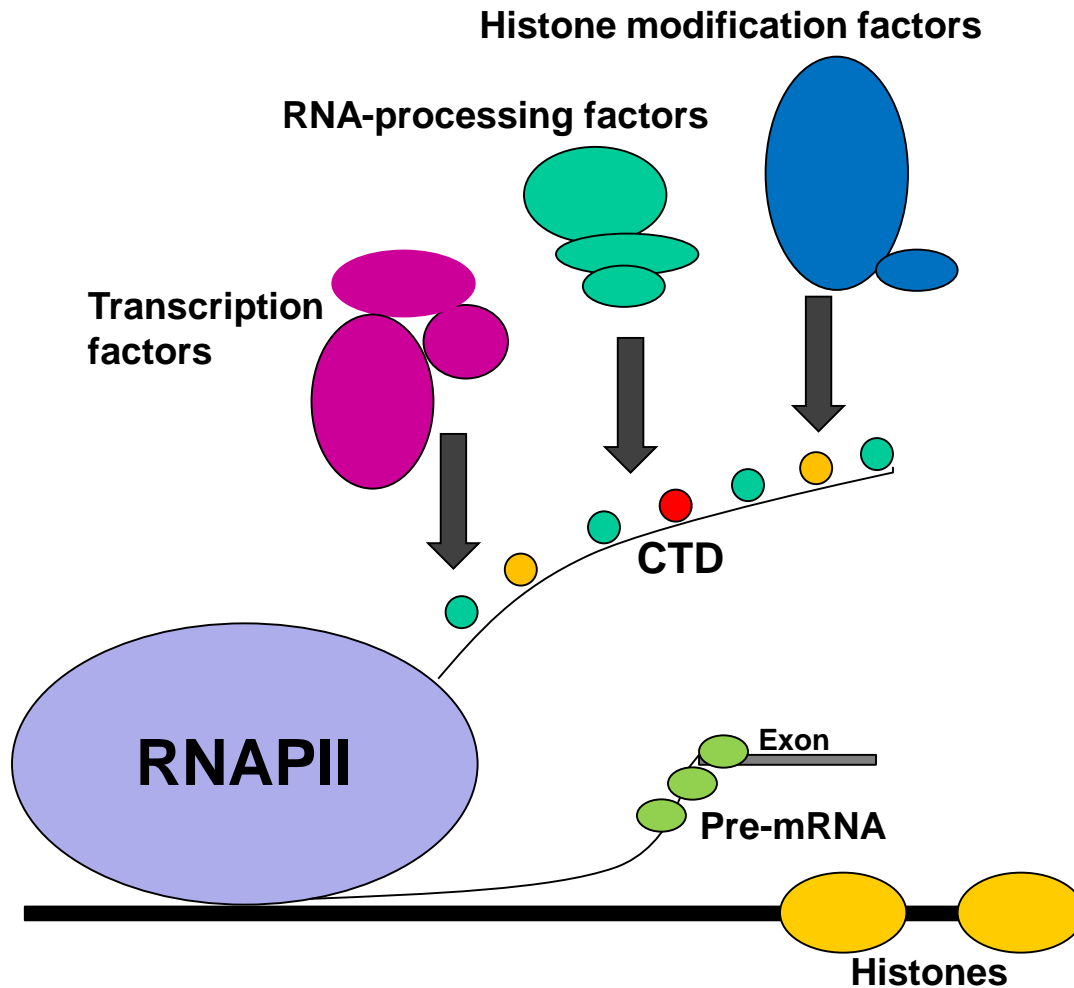
X 52 repeats in mammals
 [minus the changes in the non-consensus repeats (Figure 2)]

X 26 repeats in yeast

Roles of CDKs in the CTD phosphorylation (CTD code)



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

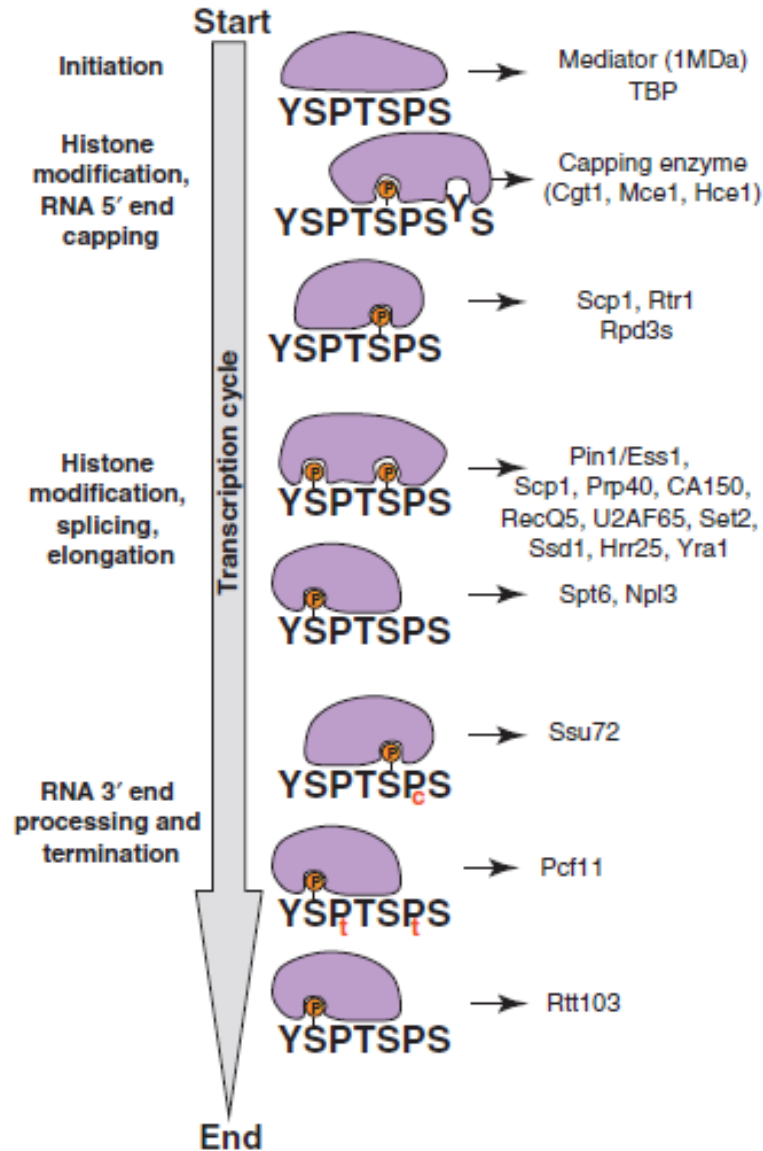


Phosphorylation of the CTD mediates:

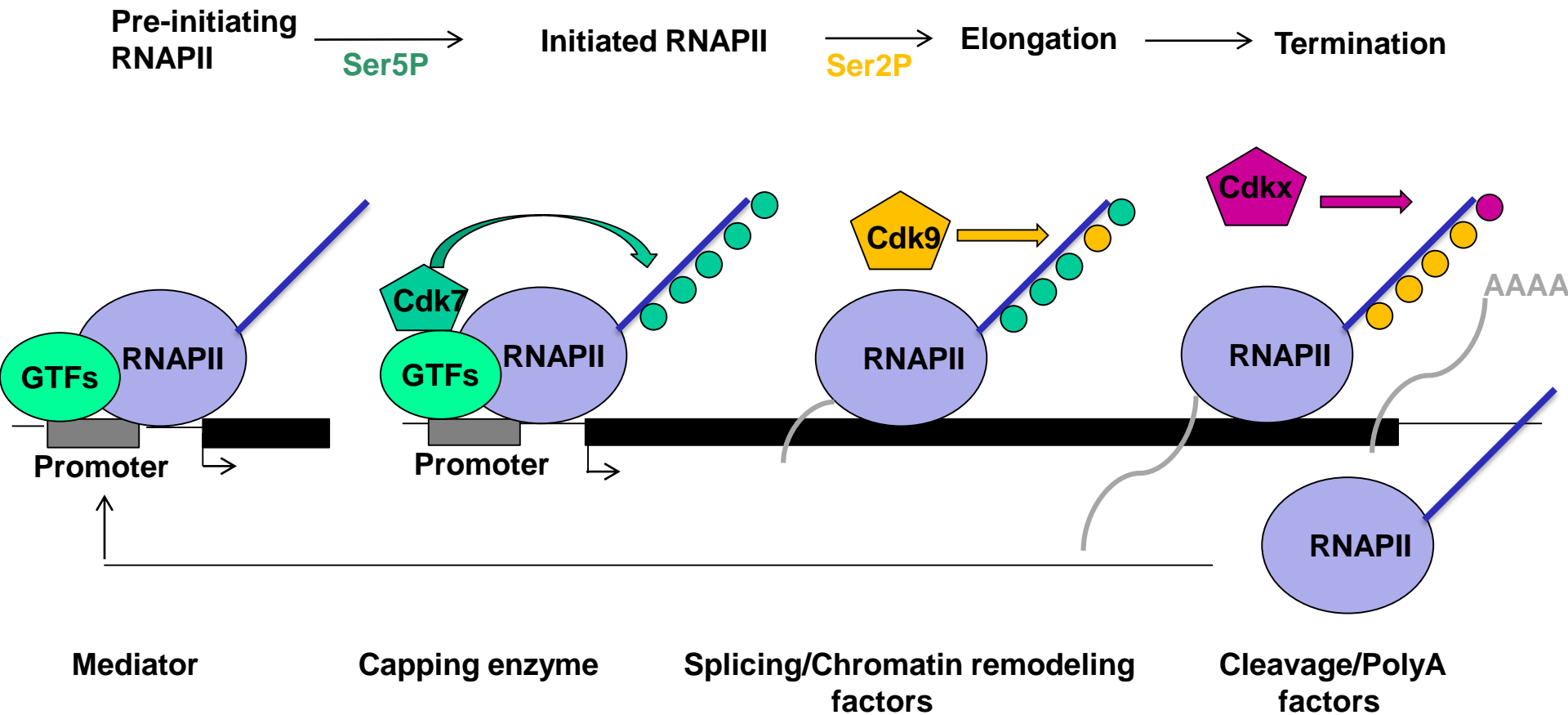
- Transcription
- mRNA-processing
- Chromatin modifications
- RNA export
- Transcription-coupled genome stability

CTD code “readers”

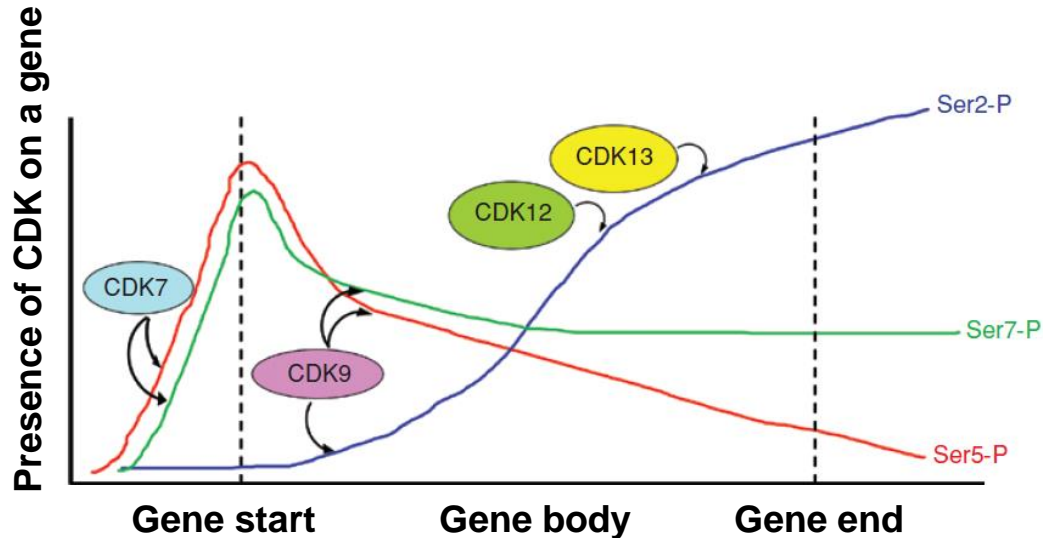
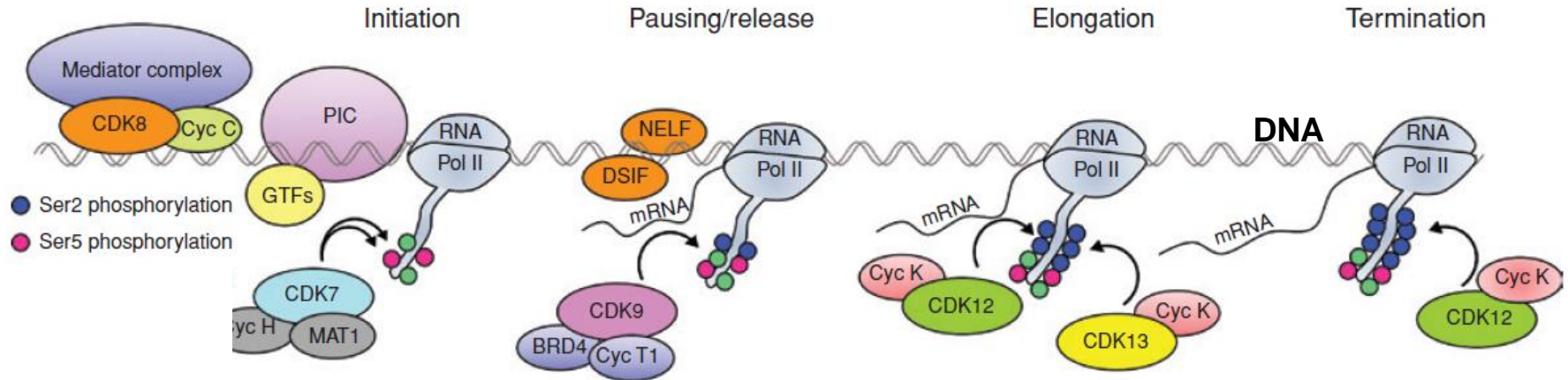
(a) *Protein-coding genes*
(yeast and mammals)



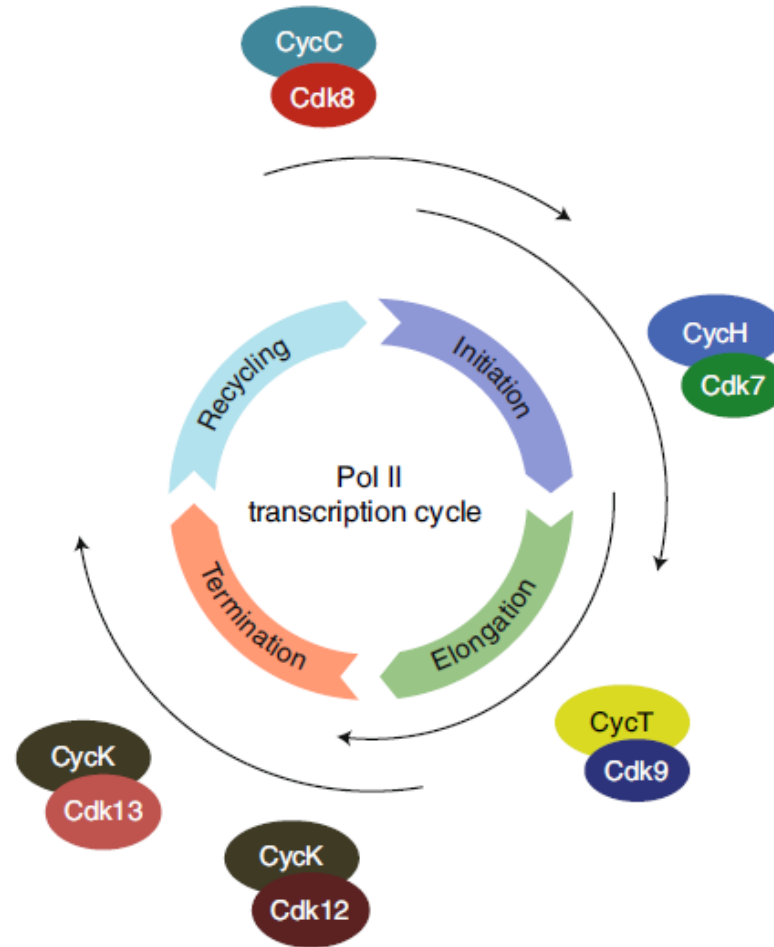
For the regulation of transcription cycle the phosphorylations of the CTD by the Cyc/Cdks are essential



Cyclin/CDKs play a major role in regulation of transcription



Cyclin/CDKs play a major role in regulation of transcription



A parallel to cell cycle regulation; i.e. each stage of transcription is regulated by a different Cyclin/CDK pair

In comparison to cell cycle CDK, the individual CDKs are NOT redundant

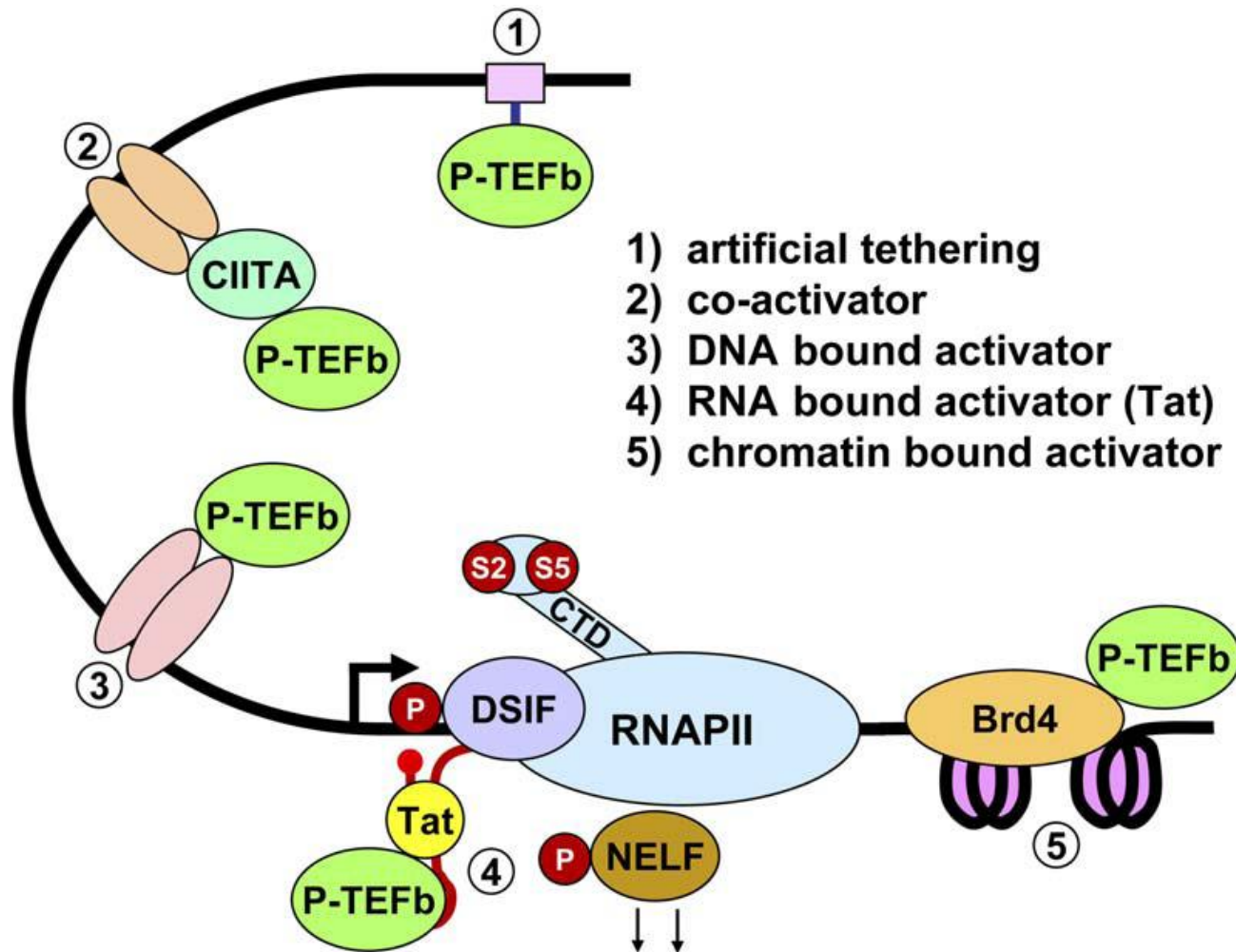
Major differences between Transcription and Cell Cycle Cyc/CDK complexes

Transcription Cyc/CDKs complexes:

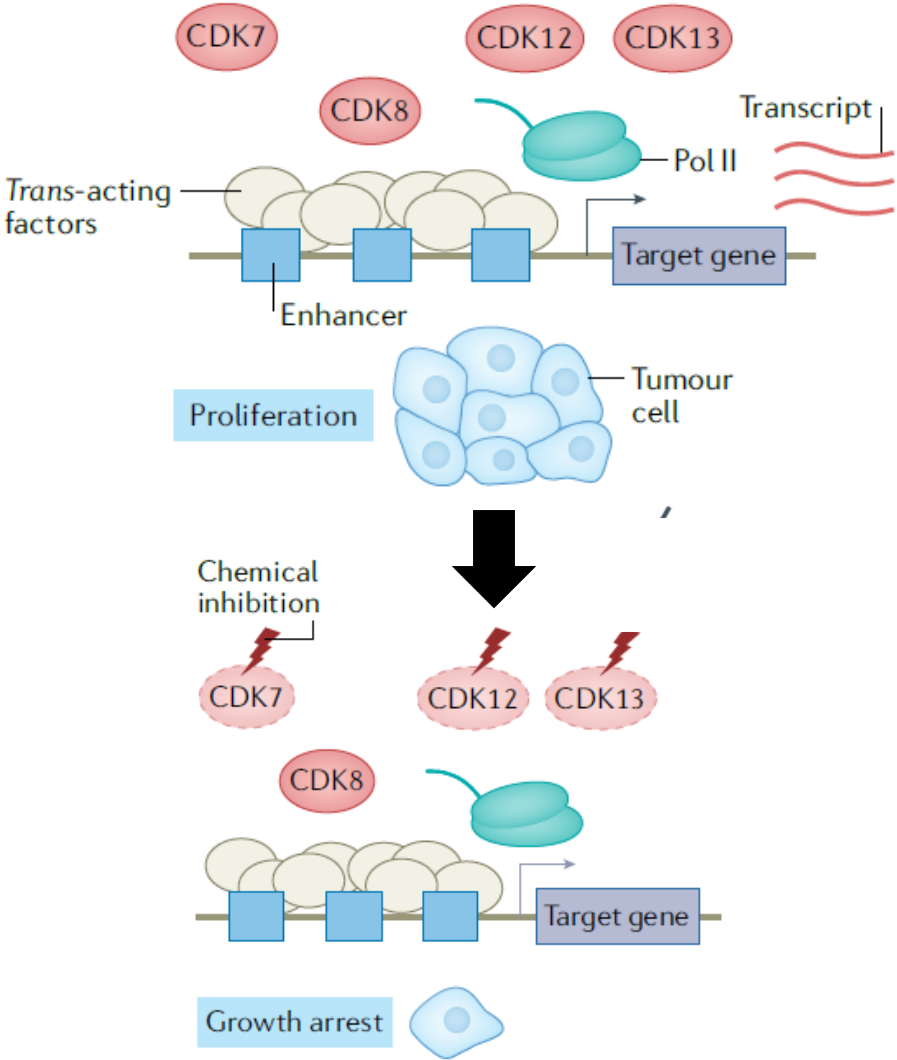
- 1) Cyclin levels in cells do not oscillate
(CDKs need to be constantly active for basal transcription)
- 2) Regulated at the level of recruitment to specific gene

Ad 2) Examples of recruitment of P-TEFb (Cdk9) to genes

P-TEFb=Cdk9



Deregulation of transcription and cancer



Concept of "transcriptional addiction" of a cancer cell

Regulation of CDKs kinase activity

Kinase activity is crucial for many cellular processes

Deregulation of kinase activity results in a disease



The kinase activity is strictly regulated

Regulation of kinase activity of CDK complexes

Overview:

Activation of CDK kinase activity:

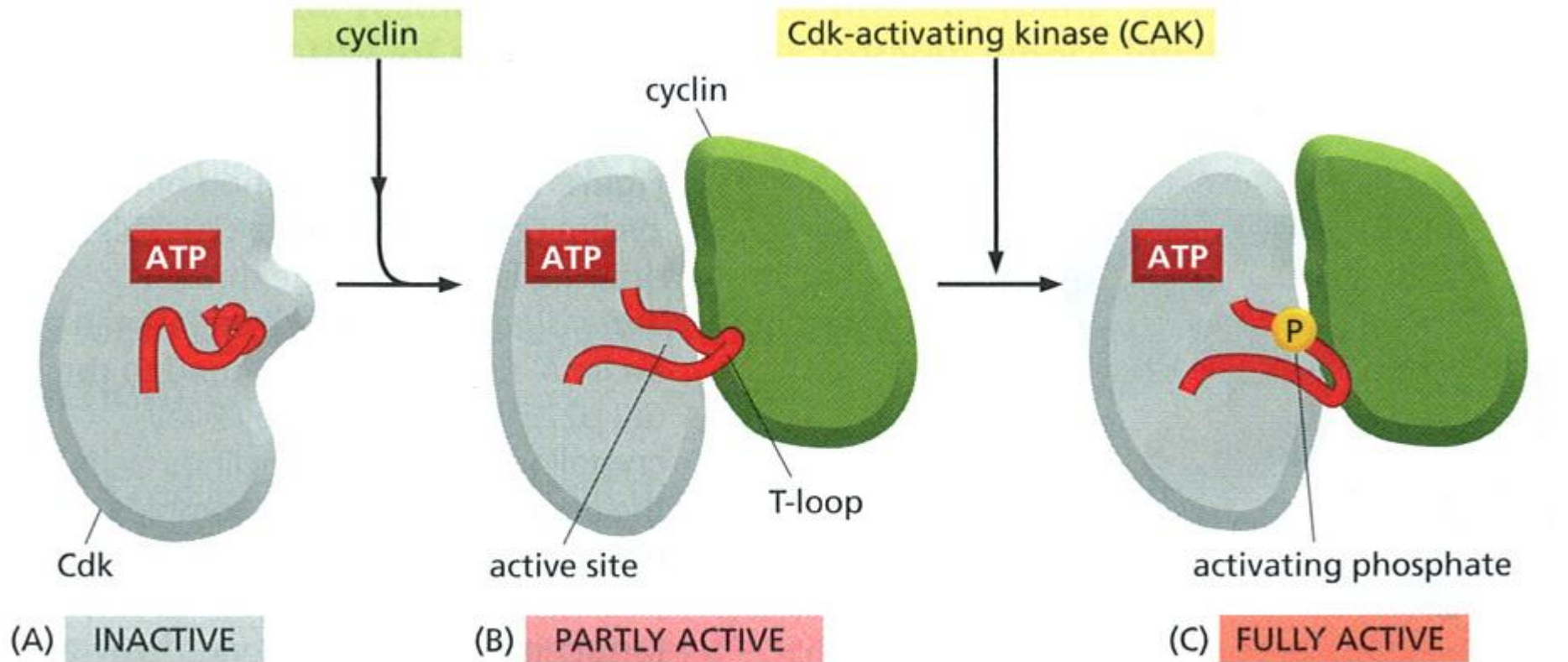
- Association of CDK with various Cyclin subunits
- Phosphorylation of threonine in the “T-loop” of CDK
- Degradation of CDK inhibitor proteins by ubiquitination and proteolysis

Inhibition of CDK kinase activity:

- Binding of CDK inhibitor proteins to Cyc/CDK complexes
- Inhibitory phosphorylation of CDK
- Ubiquitination 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

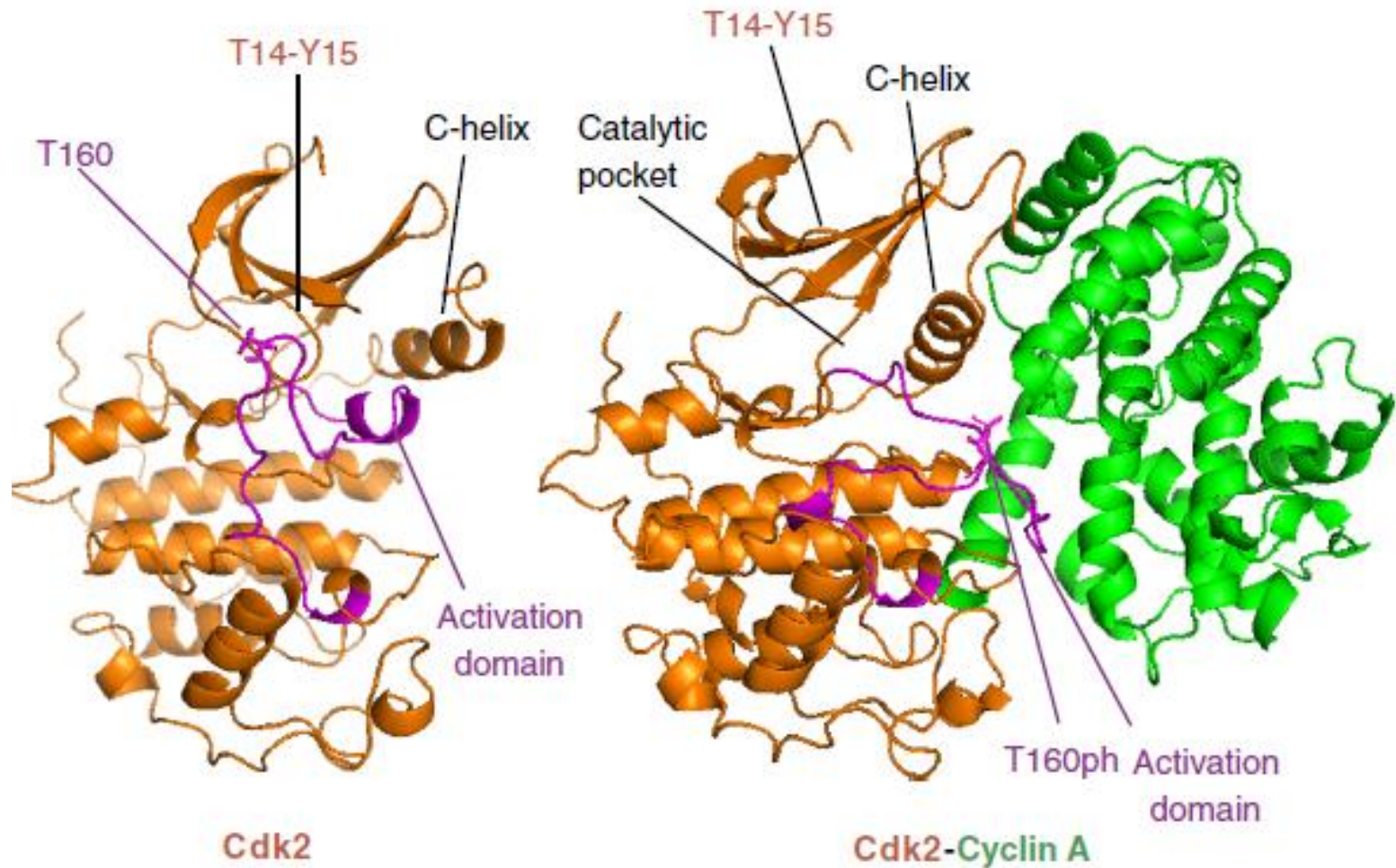


T-loop blocks active site
(active site=ATP binding site)

T-loop moves out of the active site

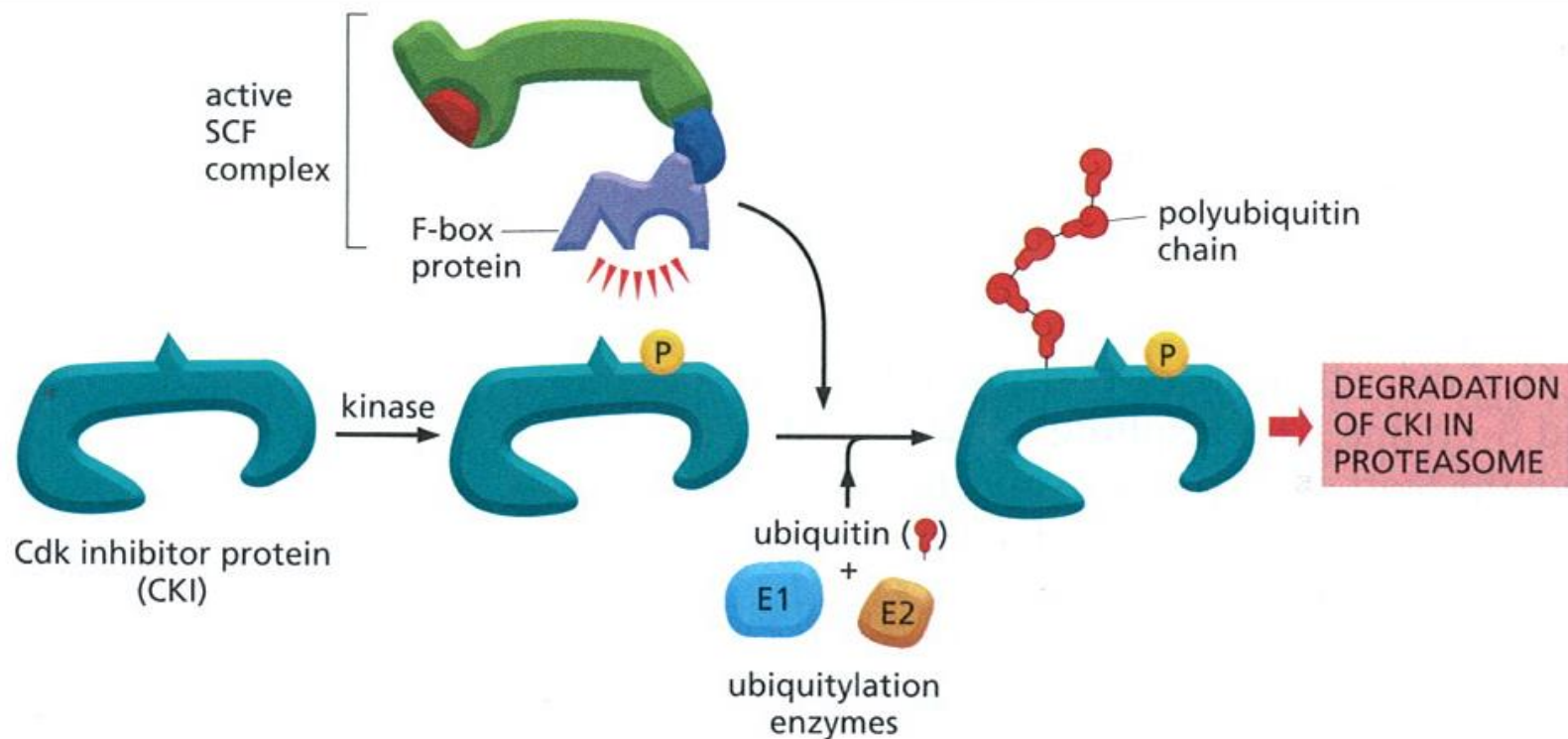
P-T-loop improves binding of substrate

Activation of Cdk kinase activity-Cdk2-Cyclin A



Activation of CDK kinase activity:

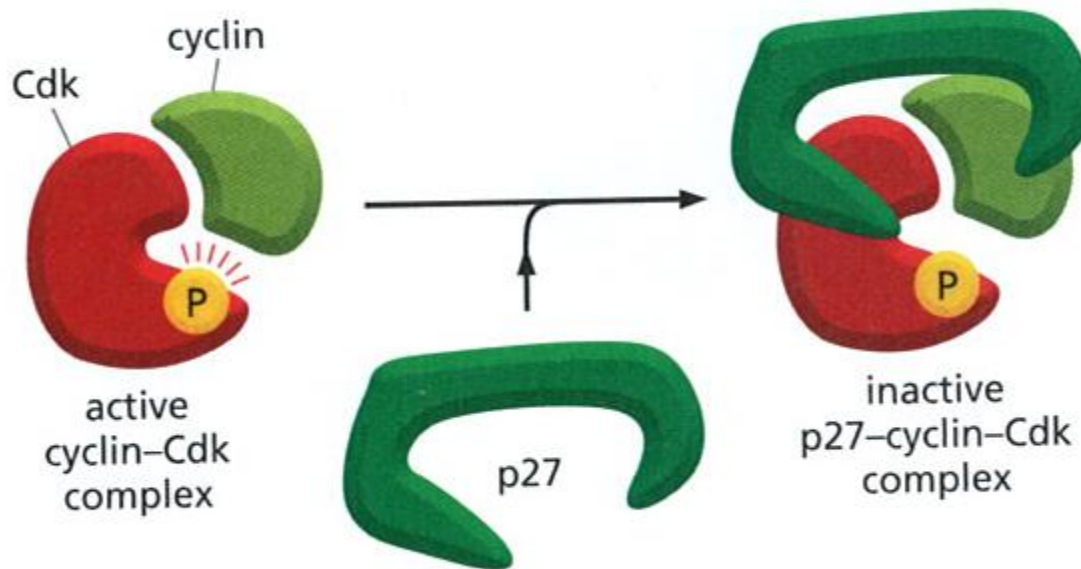
-Degradation of CDK inhibitor proteins by ubiquitination and proteolysis



Cell cycle-dependent phosphorylation of CDK inhibitor is a “mark” for recognition by SCF ubiquitin ligase, ubiquitylation and degradation, rendering Cyc/CDK complex more active

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)

p27 (mammals)

p21 (mammals)

p16 (mammals)

suppresses Cdk1 activity in G₁; phosphorylation by Cdk1 at the end of G₁ triggers its destruction

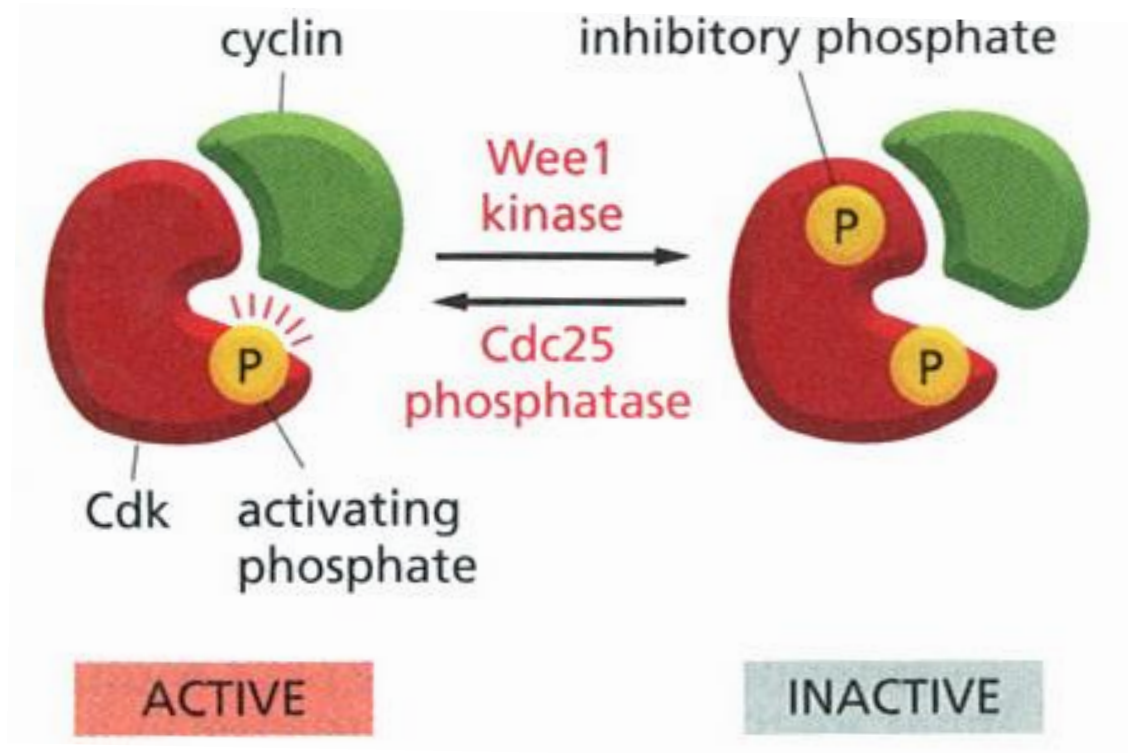
suppresses G₁/S-Cdk and S-Cdk activities in G₁; helps cells withdraw from cell cycle when they terminally differentiate; phosphorylation by Cdk2 triggers its ubiquitylation by SCF

suppresses G₁/S-Cdk and S-Cdk activities following DNA damage

suppresses G₁-Cdk activity in G₁; frequently inactivated in cancer

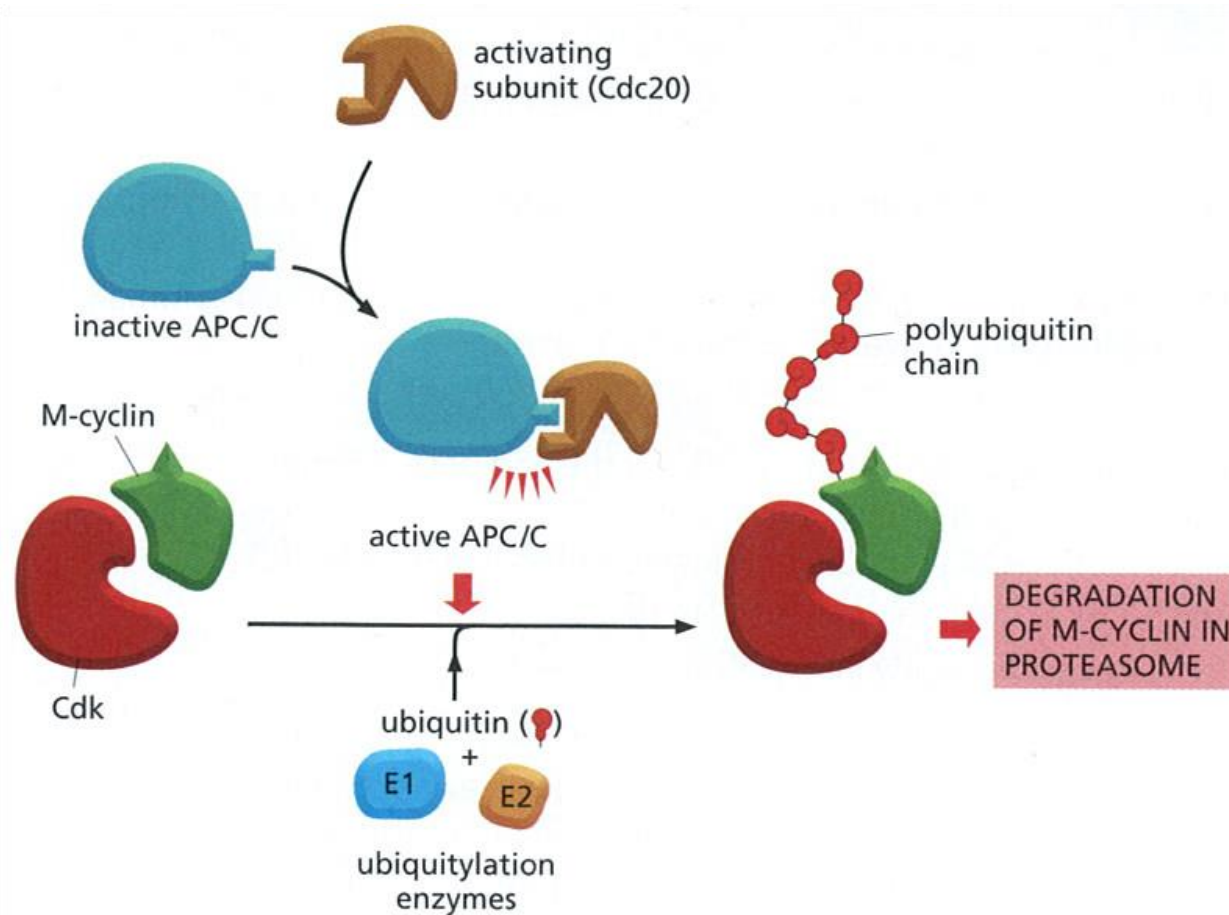
Inhibition of CDK kinase activity:

-Inhibitory phosphorylation of CDK



Inhibition of CDK kinase activity:

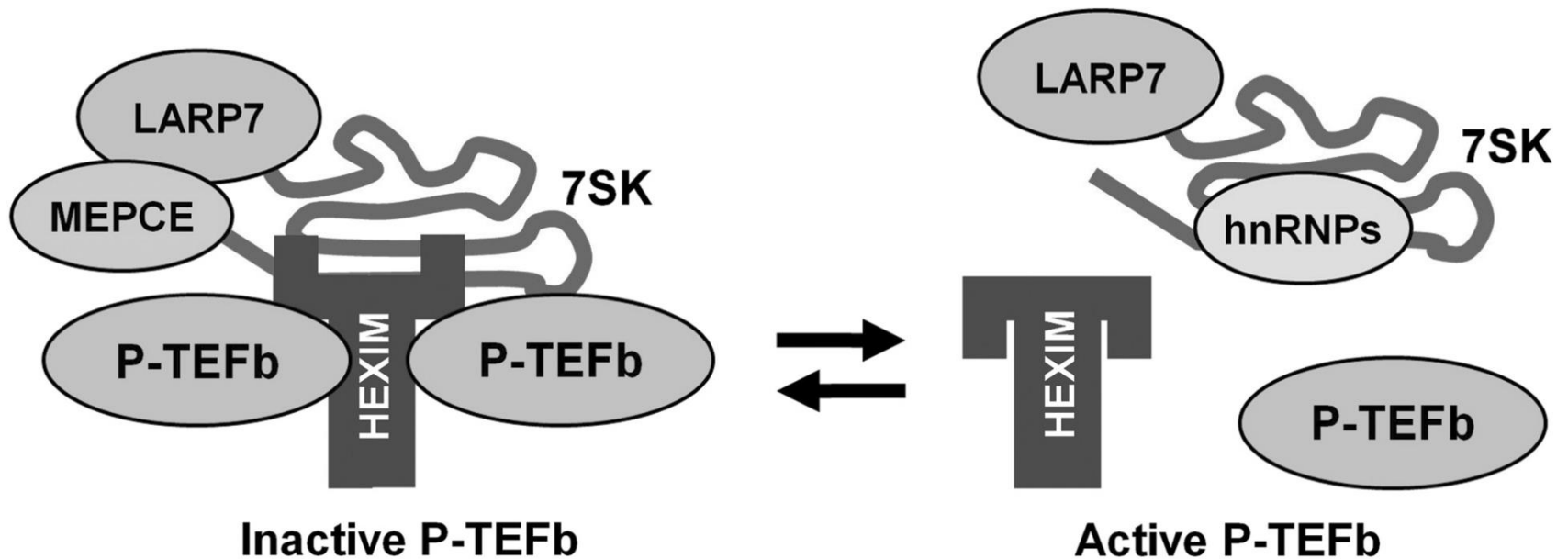
-Ubiquitination and degradation of Cyclin by proteasome



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



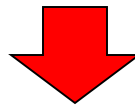
P-TEFb=CDK9

The kinase activity of CDK9 is inhibited by binding to several proteins and small nuclear RNA, 7SK snRNA

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

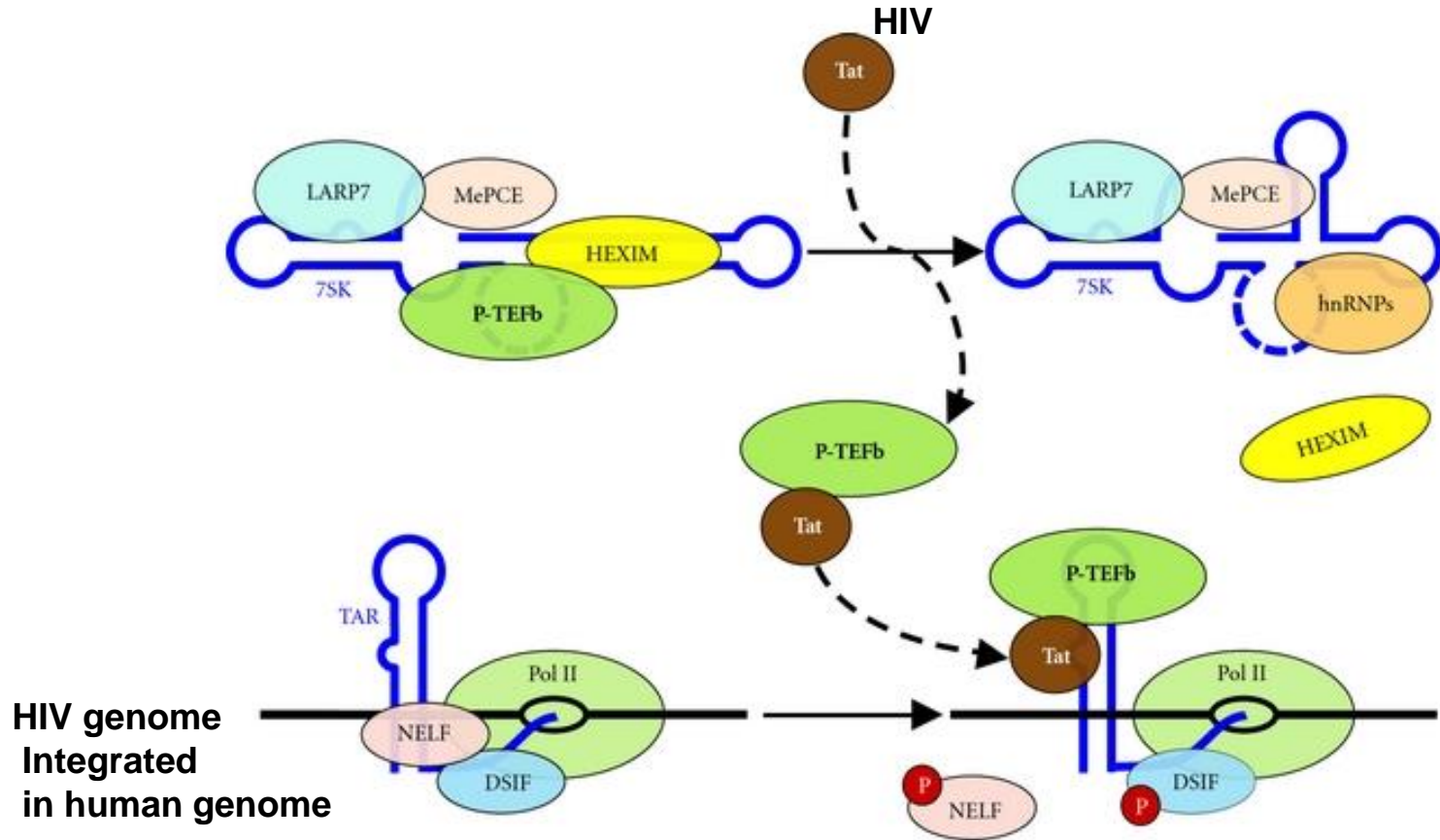
-HIV transcription- HIV specific Tat protein “steals” CDK9 from its cellular complex to transcribe HIV genome

-Cancer - aberrant kinase activity of cell cycle and transcriptional CDKs (CDK4,6,7, 9,12)



defective cell cycle and transcription

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

Alterations of CDKs and their regulators in cancer

CDKs and their regulators play a role as oncogenes and tumor suppressors



Their alteration leads to a development of cancer

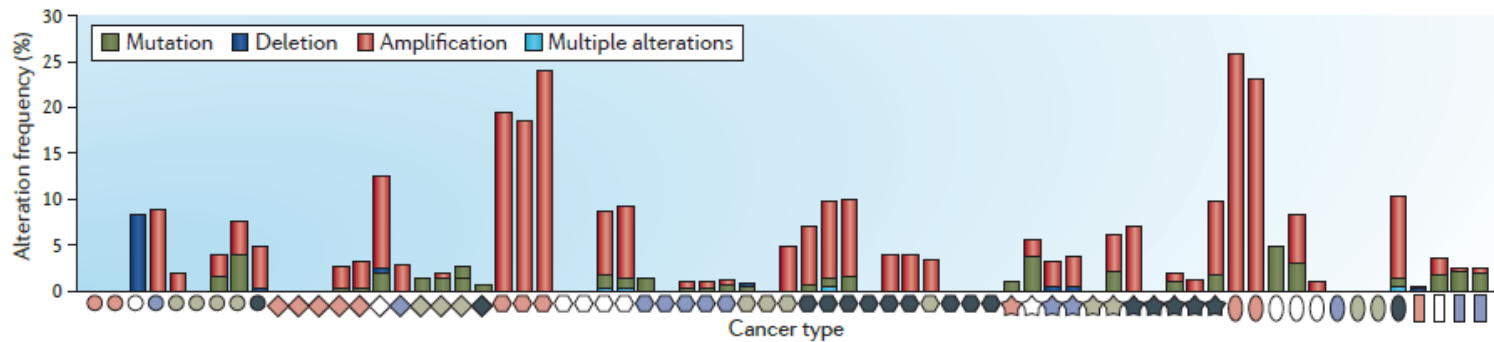
Oncogene=gene that drives a cancer

Tumor suppressor=gene that prevents a cancer

Cell cycle CDKs/Cyclins are often amplified in various cancers (play a role of oncogenes)

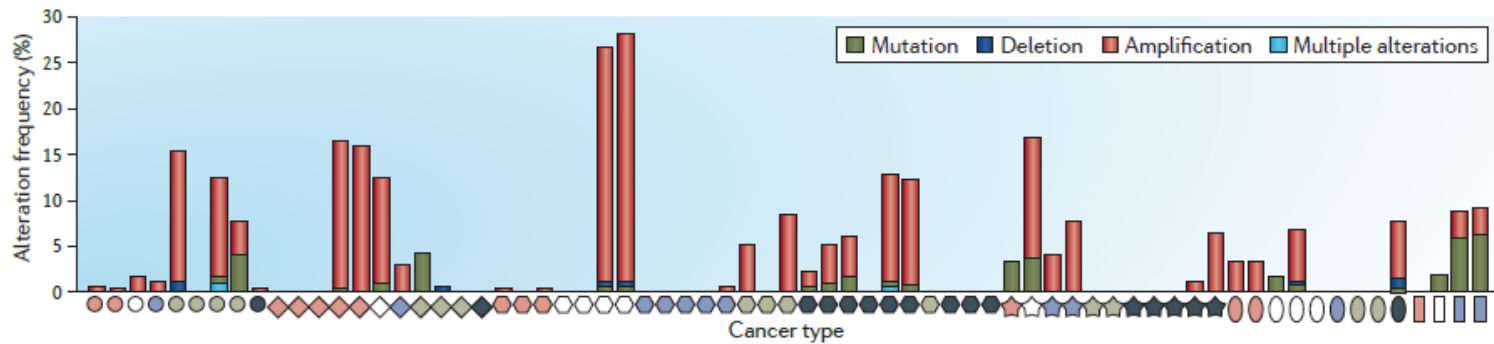
CDK4/CDK6

CDK4 and CDK6



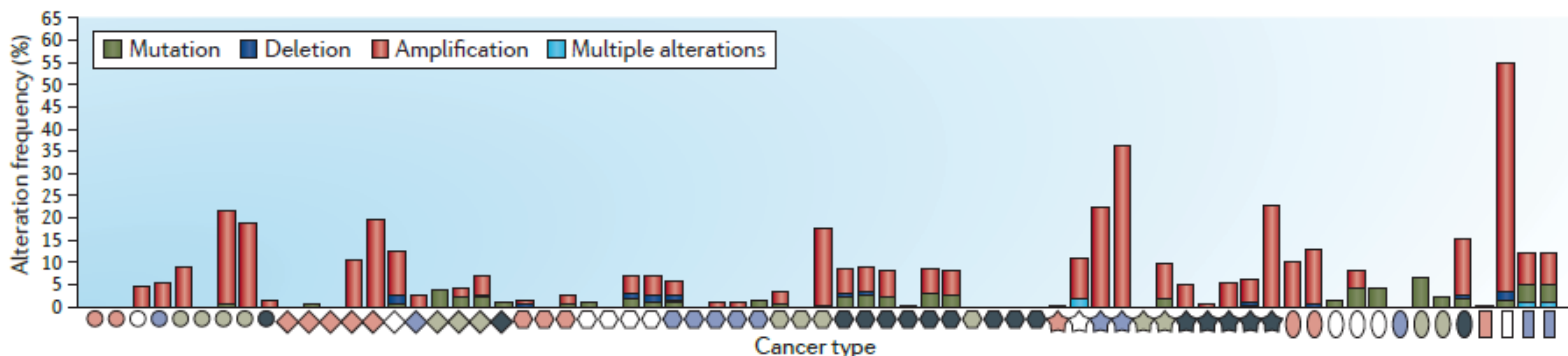
Cyclin D

CCND1



Cyclin E

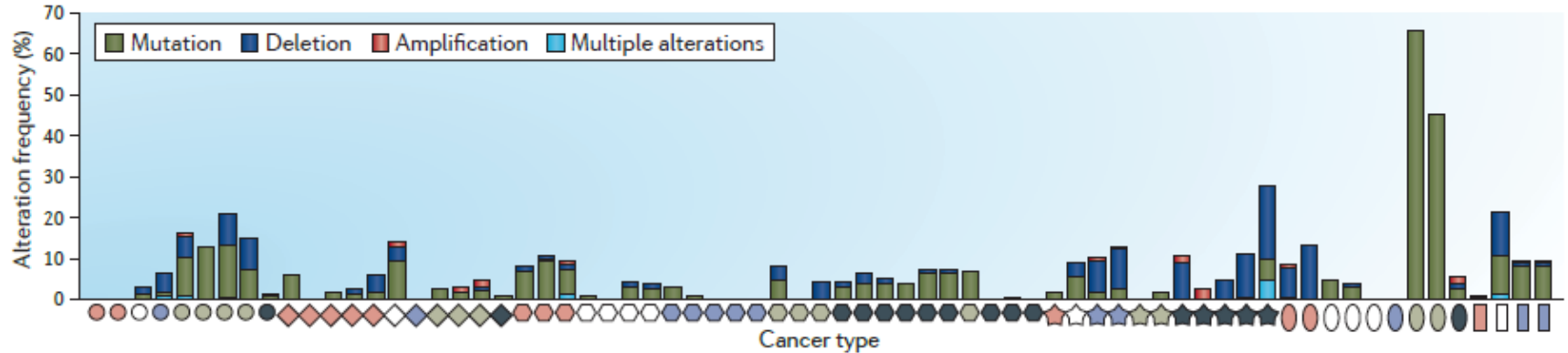
CCNE1 and CCNE2



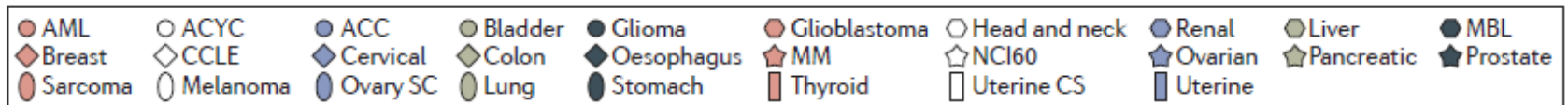
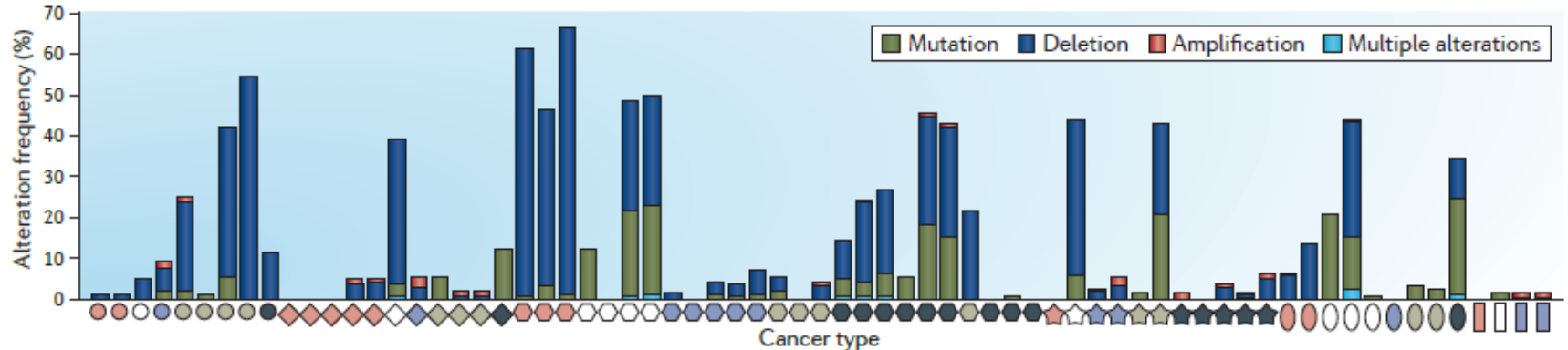
AML	ACYC	ACC	Bladder	Glioma	Glioblastoma	Head and neck	Renal	Liver	MBL
Breast	CCLC	Cervical	Colon	Oesophagus	MM	NCI60	Ovarian	Pancreatic	Prostate
Sarcoma	Melanoma	Ovary SC	Lung	Stomach	Thyroid	Uterine CS	Uterine		

Inhibitors/regulators of cell cycle CDKs/Cyclins are often deleted or mutated in various cancers (play a role of tumor suppressors)

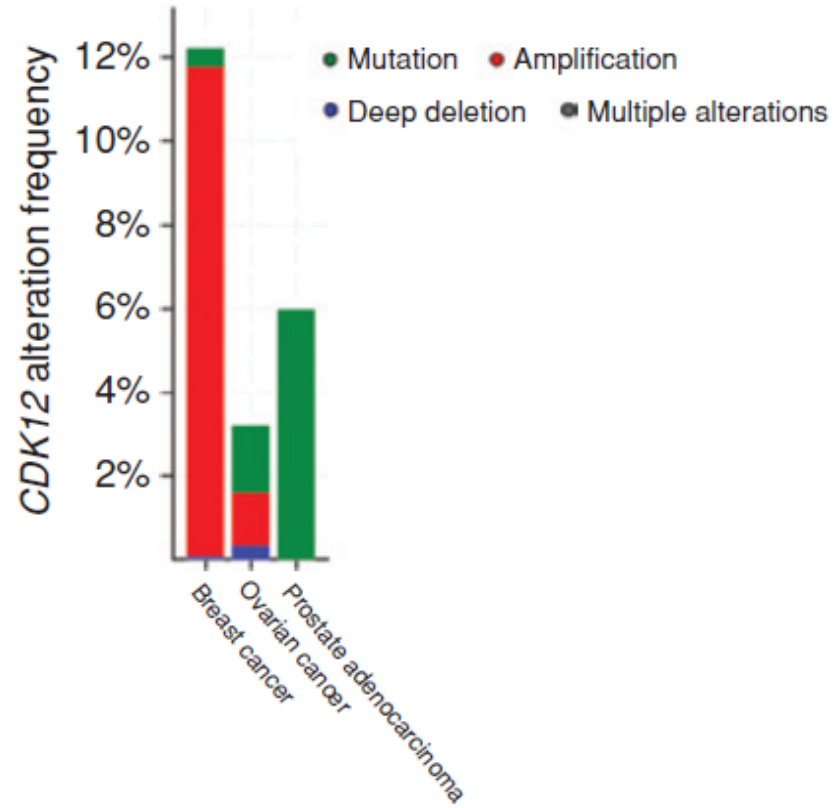
RB1 RB1



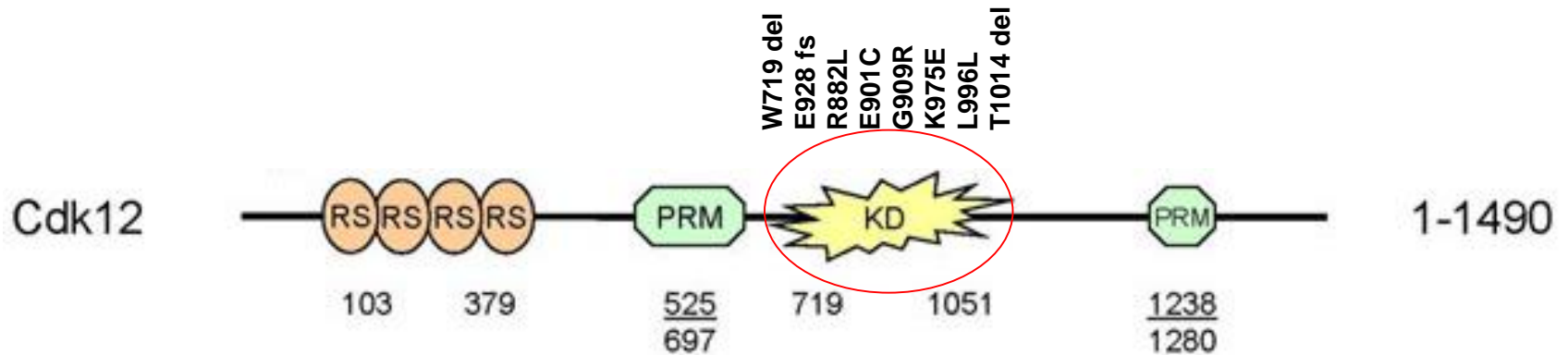
p21 CDKN2A



Transcriptional CDK12 can be either amplified or mutated in various cancers (oncogene / tumor suppressor)



Transcriptional CDK12 is mutated in prostate and ovarian cancer



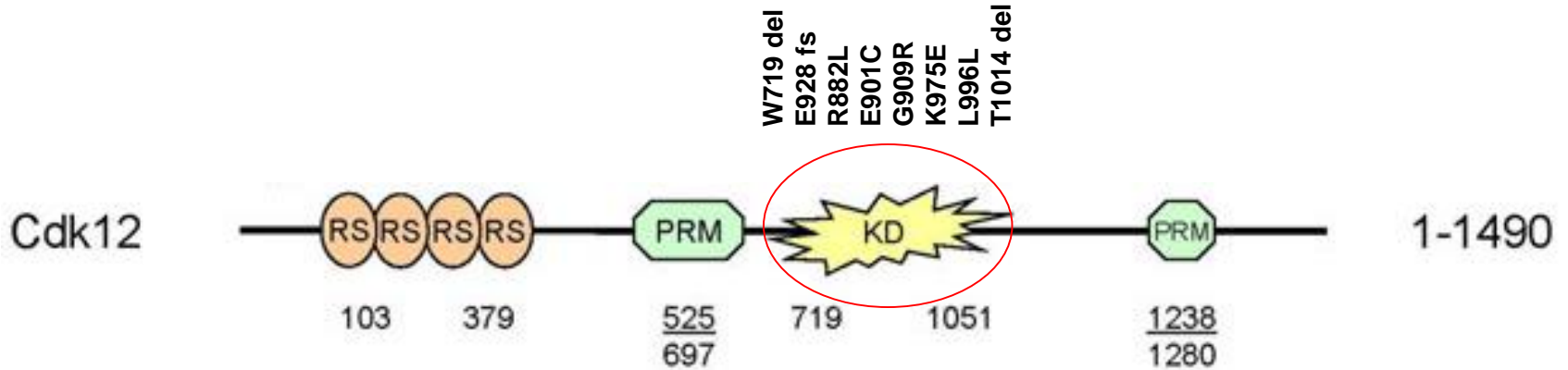
KD=kinase domain

The mutations lead to the aberrant kinase activity and defective transcription of certain genes important to preventing cancer (like DNA damage response genes-BRCA1, BRCA2 etc)

CDK12 proposed to be a novel tumor suppressor

Alterations in CDKs/Cyclins can be used as biomarkers for cancer treatment

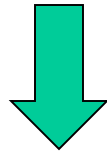
CDK12 mutations in ovarian cancer/breast:



Indication for treatment with olaparib (PARP inhibitor)

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

- **CDKs or cyclins are often amplified, overexpressed or activated in cancers (CDK4, CDK6, Cyclin E, Cyclin D) (CDK12, CDK9, CDK7, CDK8)**



Use of chemical inhibitors

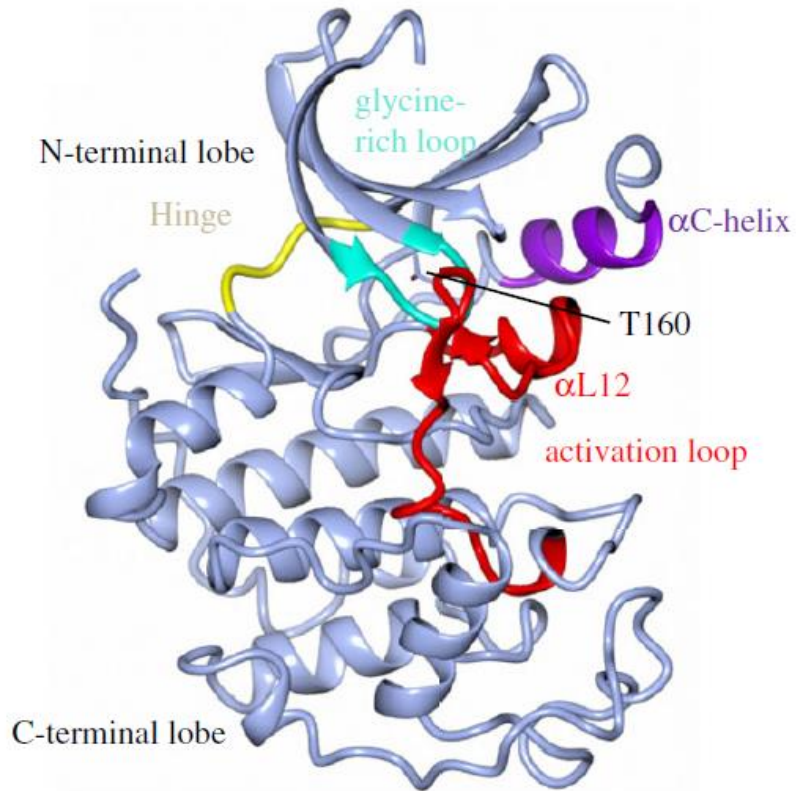
Issues with kinase inhibitors

- **Selectivity**-activity towards other kinases
(ideally targeting only 1 kinase)
- **Potency**-concentration needed for inhibition of the kinase
(IC 50 ideally in low nM)

In practice: chemical inhibitors almost always inhibit other kinases

Sometimes the outcome depends on inhibition of proper spectrum of kinases in a particular tumor

Kinase domain structure and similarities within CDK family

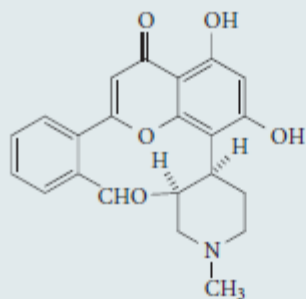


CDK2



Overlap of CDK2/CDK6/CDK7/CDK16

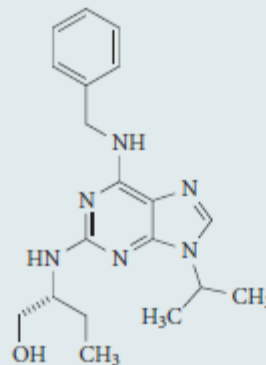
CDK inhibitors often have low selectivity within CDK family



Flavopiridol

IC₅₀

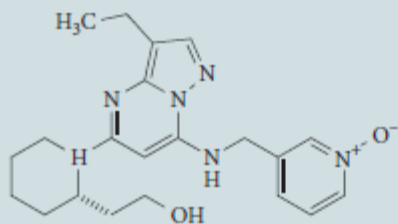
- CDK1: 30 nM
- CDK2: 170 nM
- CDK4: 100 nM
- CDK5: 170 nM
- CDK7: ND
- CDK9: 20 nM



Roscovitine

IC₅₀

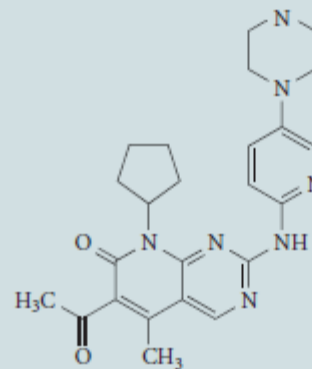
- CDK1: 330 nM
- CDK2: 220 nM
- CDK4: >10 μM
- CDK5: 270 nM
- CDK7: 800 nM
- CDK9: 230 nM



Dinaciclib

IC₅₀

- CDK1: 3 nM
- CDK2: 1 nM
- CDK4: ND
- CDK5: 1 nM
- CDK7: ND
- CDK9: 4 nM

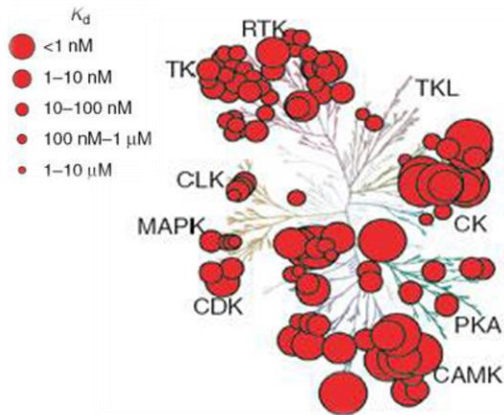


PD-0332991

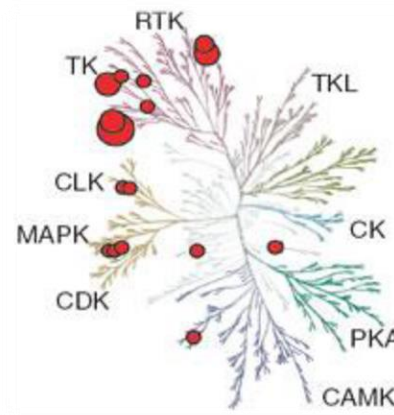
IC₅₀

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

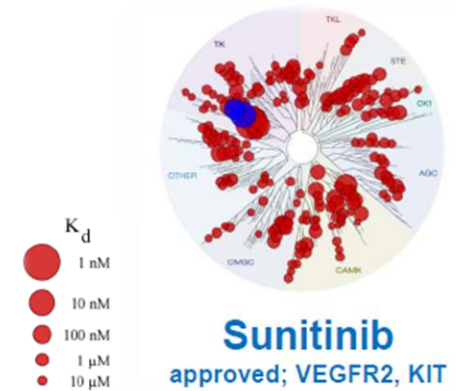
Other protein kinase inhibitors: selectivity



staurosporine



imatinib (Gleevec)
(FDA approved drug)



„quality kinase probe“ S. Knapp et al. *Nat. Chem. Biol.* 2013, 9, 3.

- potent inhibition of primary target
- at least 50 fold selectivity over other targets
- demonstrated cellular activity data



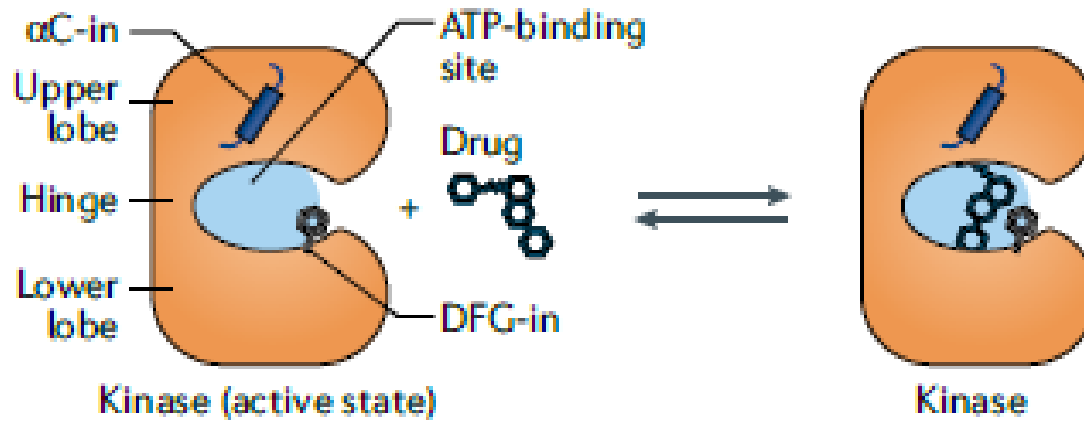
only 22 (!) inhibitors passed

Kinase inhibition modes

Inhibitors:

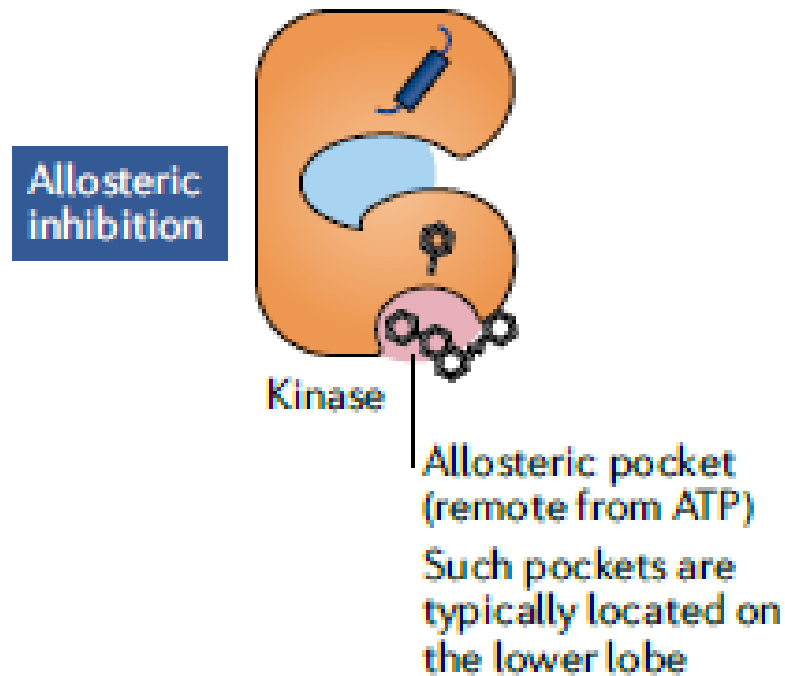
- **ATP mimic (competitive)**
- **Allosteric**
- **Covalent**
- **Degraders (PROTACs)**

Kinase inhibition modes



ATP mimic (competitive) inhibitors

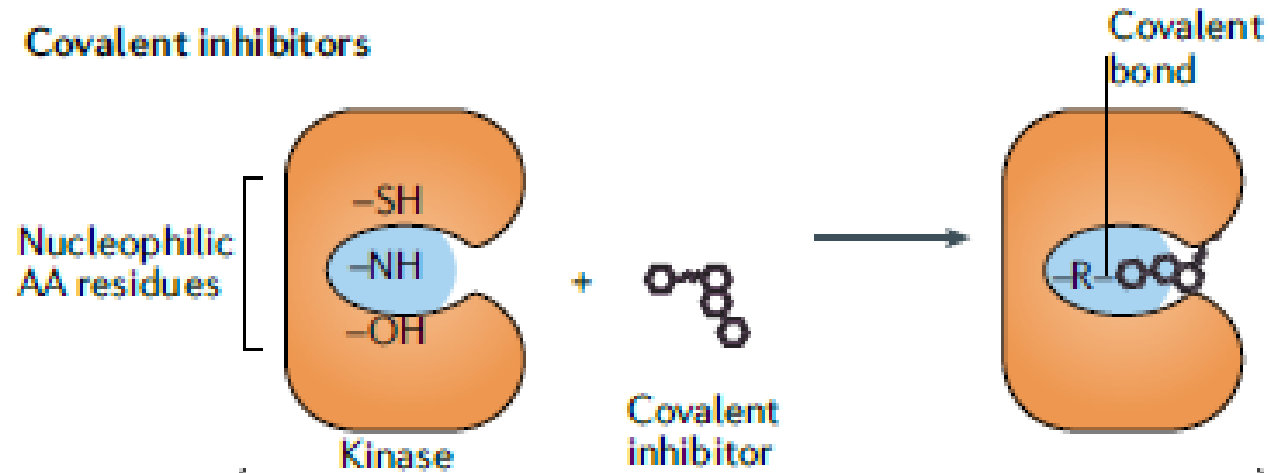
Kinase inhibition modes



allosteric inhibitors

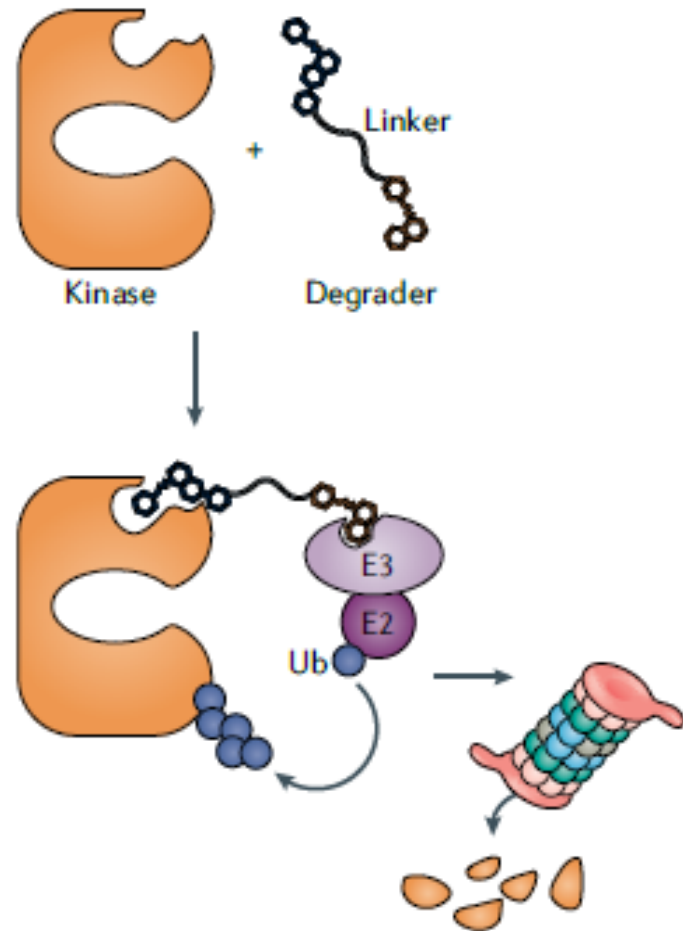
Kinase inhibition modes

Covalent inhibitors



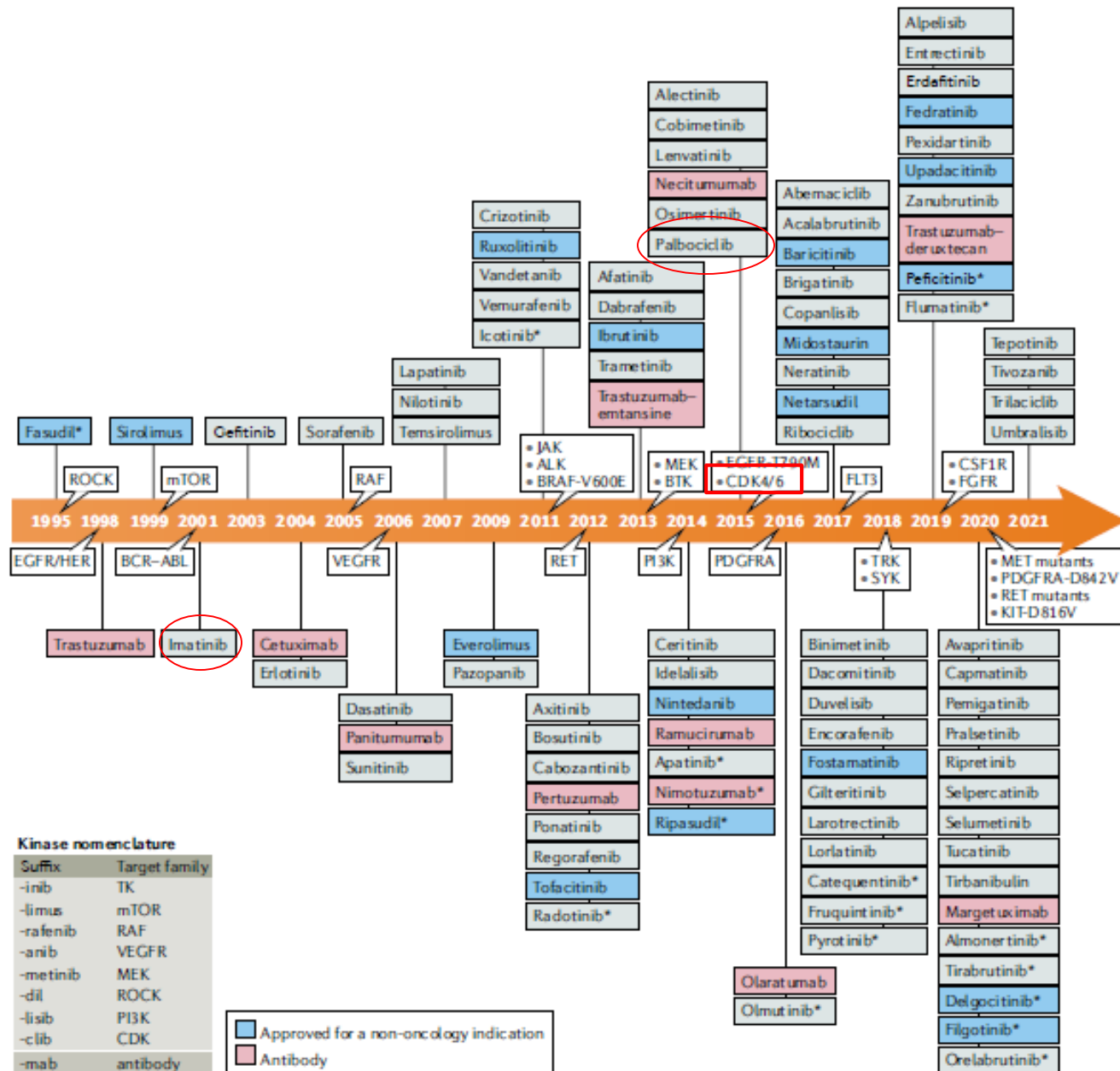
Covalent inhibitors

Kinase inhibition modes



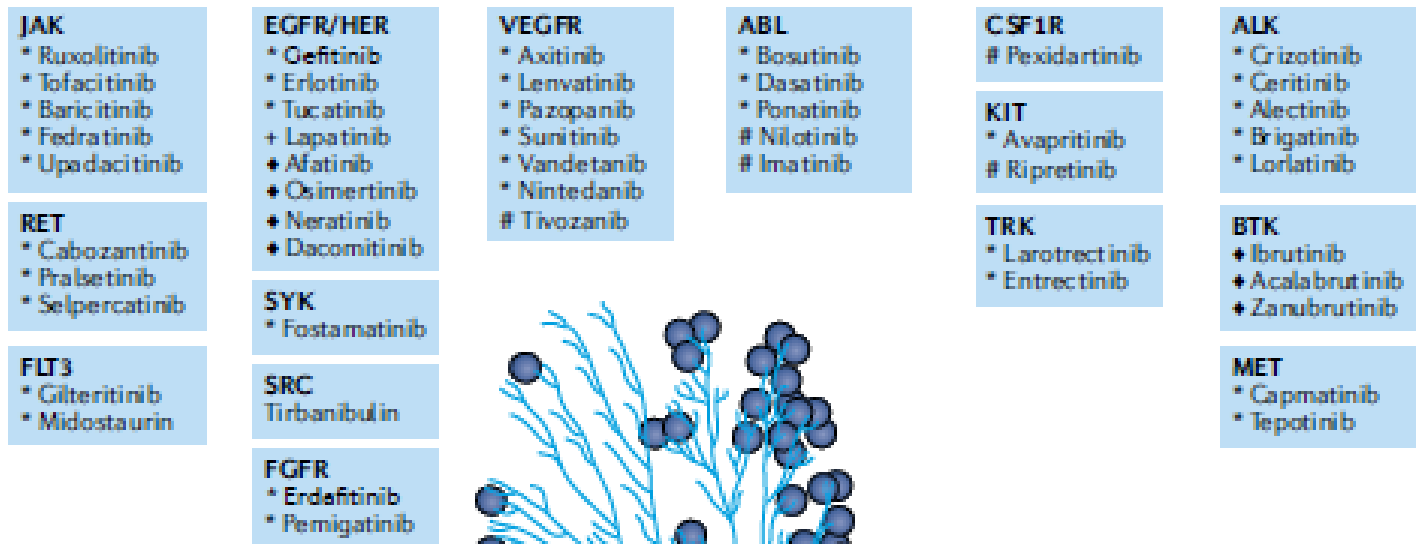
Degraders (PROTACs)

Time-line of approved kinases inhibitors



Food and drug administration (FDA) -approved kinase inhibitors mapped into the human kinome

Tyrosine kinase group



Lipid kinases

- PI3K**
- * Alpelisib
 - * Idelalisib
 - * Copanlisib
 - * Duvelisib
 - * Umbralisib



Atypical kinases

- mTOR**
- § Sirolimus
 - § Temsirolimus
 - § Everolimus

PI3K



CMGC

- CDK4/6**
- * Palbociclib
 - * Abemaciclib
 - * Ribociclib
 - * Trilaciclib

TKL

BRAF

- + Vemurafenib
- + Dabrafenib
- + Encorafenib
- # Regorafenib
- # Sorafenib

STE

- MEK1/2**
- § Trametinib
 - § Cobimetinib
 - § Binimetinib
 - § Selumetinib

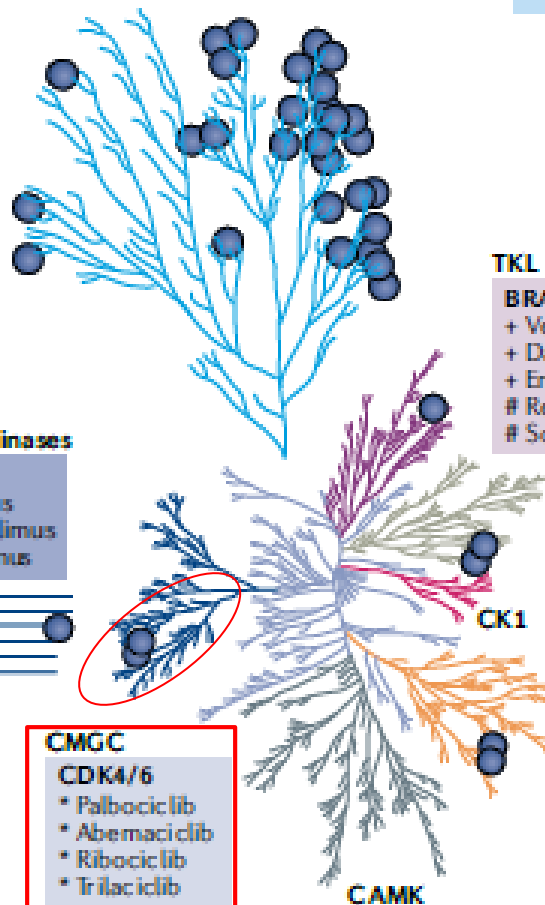
CK1

AGC

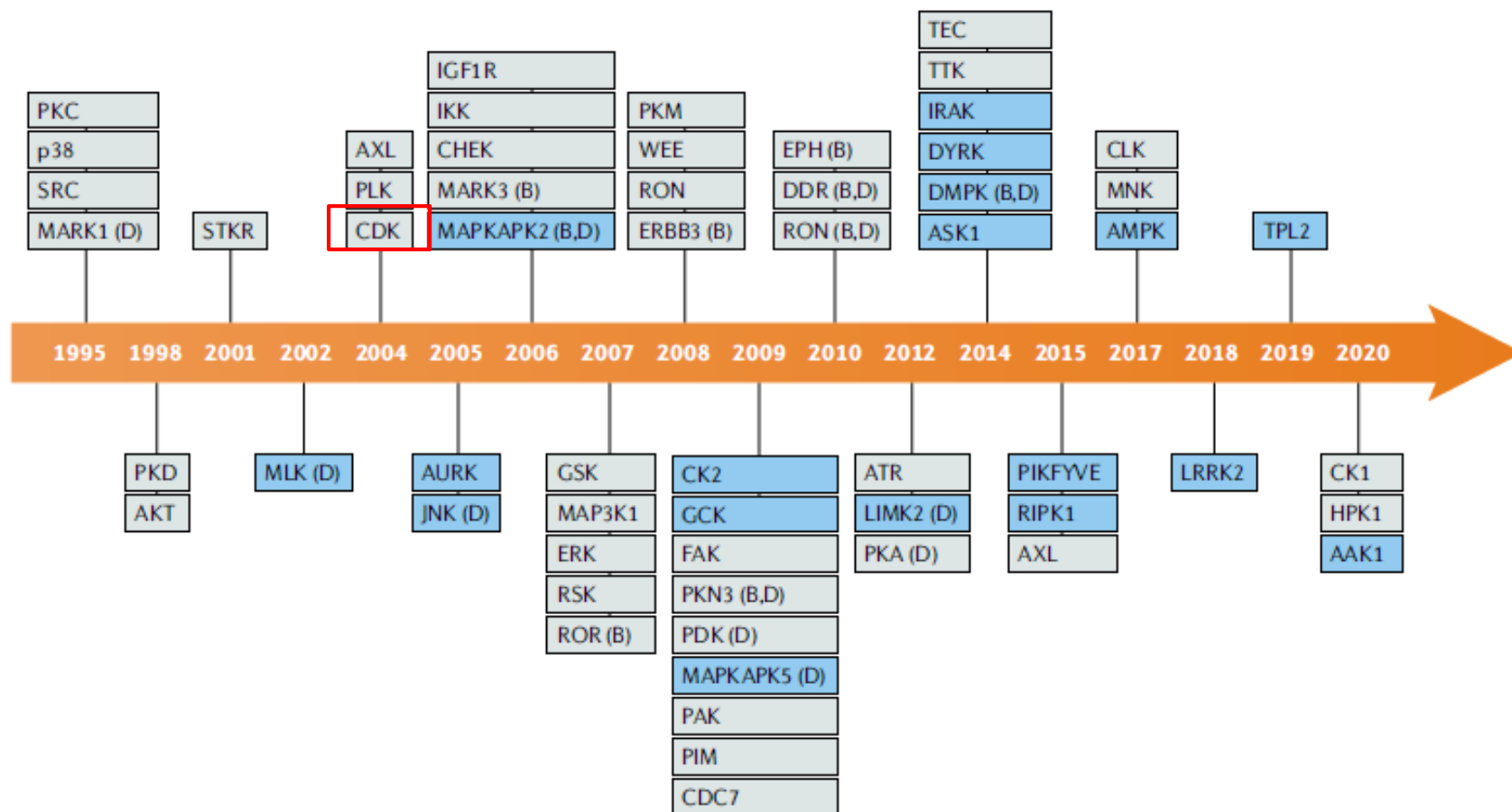
- ROCK1/2**
- * Netarsudil

Type of inhibitor

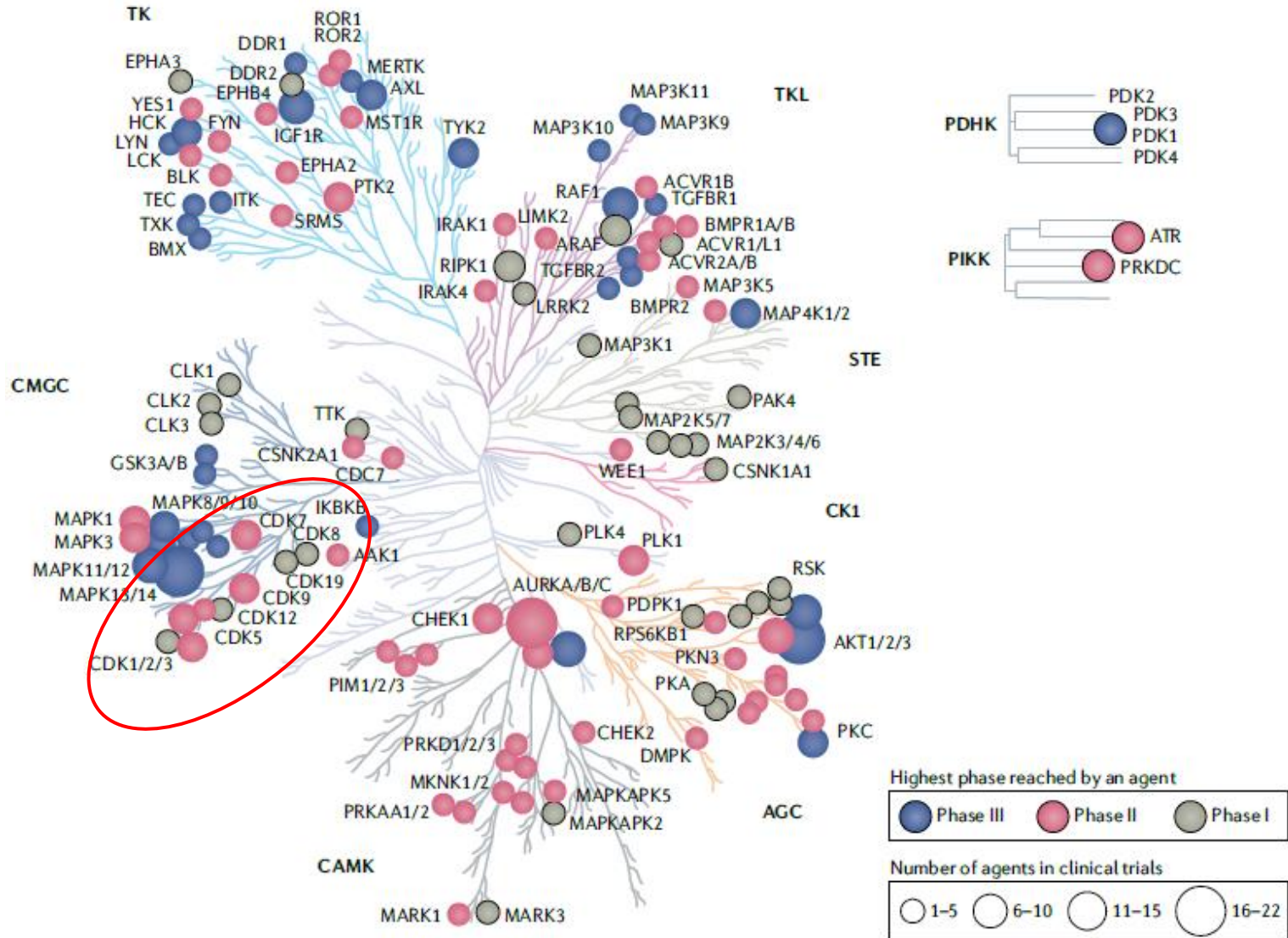
- * 1
- + 1.5
- # 2
- § 3
- + Covalent



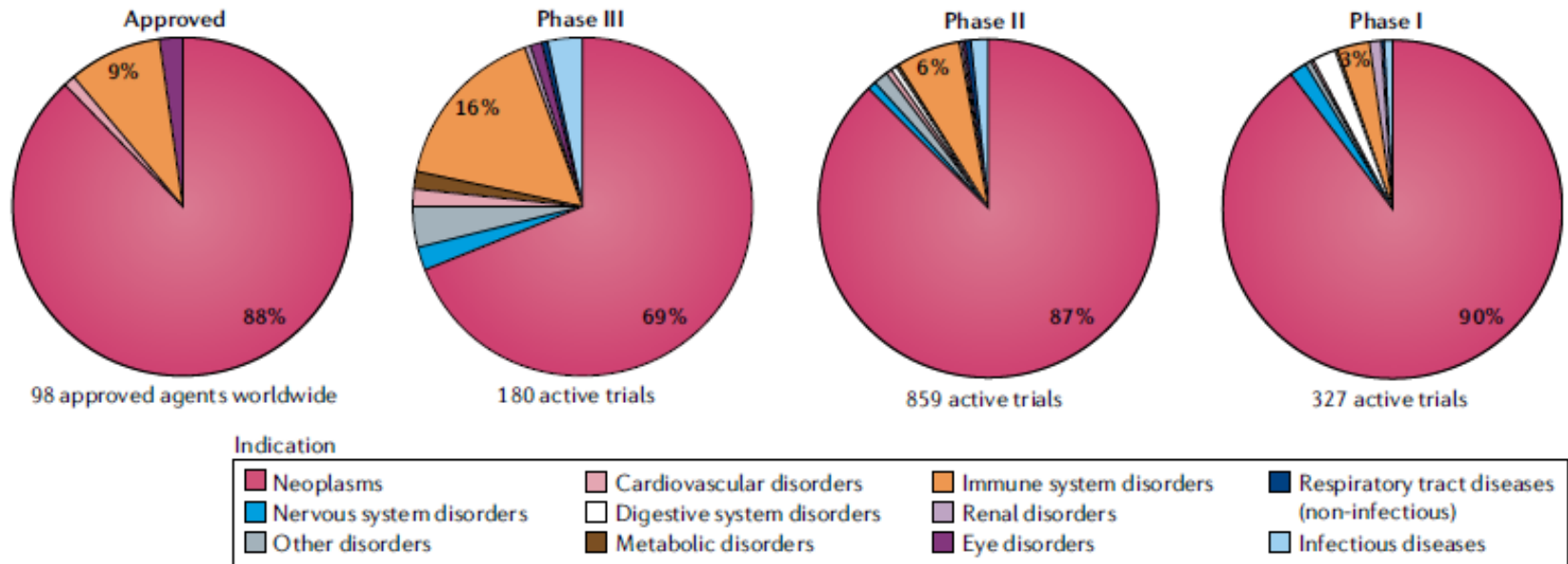
Timeline of the year for which agents with novel kinase family entered clinical trials



Kinase targets of the small molecular inhibitors in clinical trials (2021)



Indications, ongoing clinical trials and approved drugs (2021)



Cost of bringing a single drug to the market

Cost of bringing a single drug to the market

-In average 2.6 billion USD (2019), 1 billion USD (2010)

**-Top 20 pharmaceutical companies spent 60 billion USD/year
on drug development (2019)
(Czech National budget was 66 billion USD in 2018)**

**-97% of oncology drug candidates does not make it through clinical trials
to drug approval mostly due to low efficacy and high toxicity**

CANCER

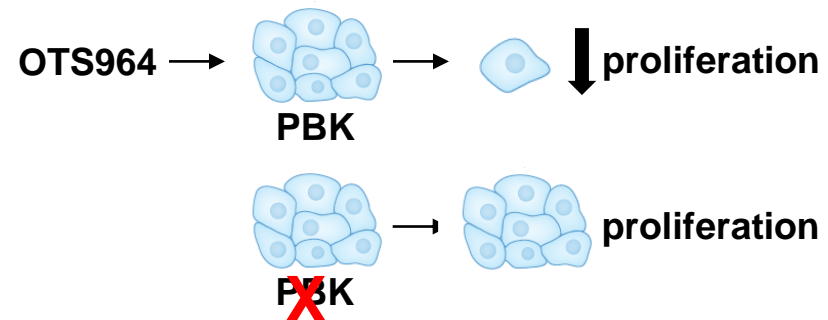
Off-target toxicity is a common mechanism of action of cancer drugs undergoing clinical trials

Ann Lin^{1,2*}, Christopher J. Giuliano^{1,2*}, Ann Palladino¹, Kristen M. John^{1,3}, Connor Abramowicz^{1,4}, Monet Lou Yuan^{1,5}, Erin L. Sausville¹, Devon A. Lukow^{1,2}, Luwei Liu^{1,2}, Alexander R. Chait¹, Zachary C. Galluzzo¹, Clara Tucker^{1,2}, Jason M. Sheltzer^{1†} (2019)

Table 1. Anticancer drugs and drug targets.

Target	Drug	No. of cancer clinical trials
CASP3	1541B	Preclinical
	PAC-1	3
HDAC6	Citarinostat	5
	Ricolinostat	10
MAPK14 (p38 α)	Ralimetinib	5
	SCIO-469	3
PAK4	PF-03758309	1
PBK (TOPK)	OTS514	Preclinical
	OTS964	Preclinical
PIM1	SGI-1776	2

Depletion of all 6 targets from cells did not stop the cancer growth!

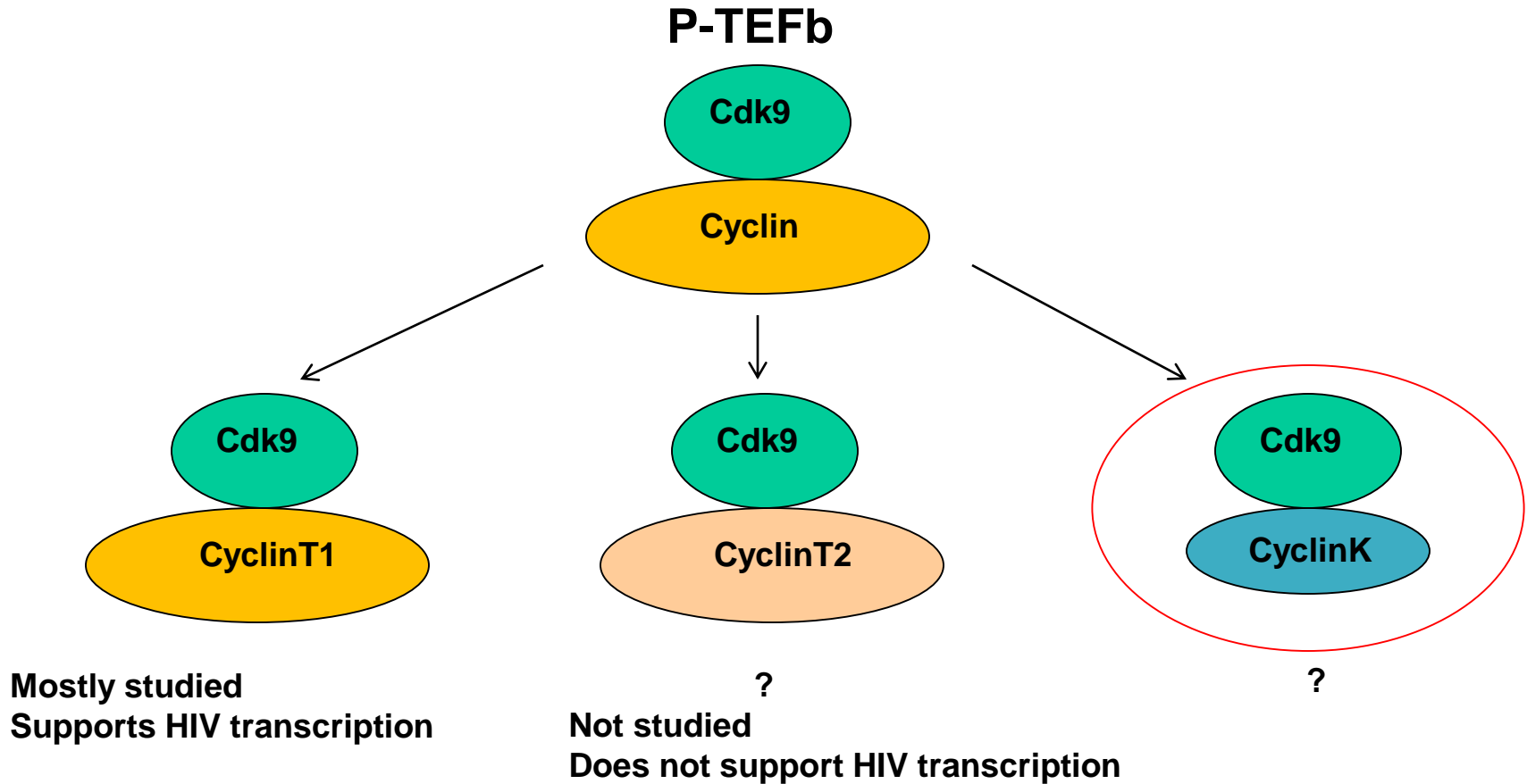


OTS964 works via inhibiting other kinase!!!
(CDK11 is bona fide target of OTS964)

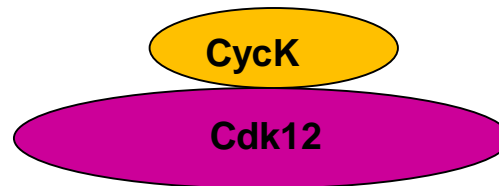
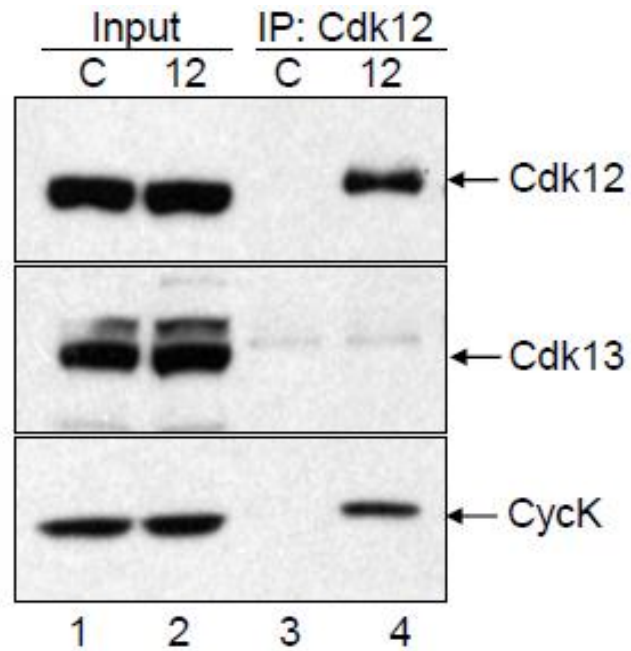
Take home message: Importance of basic science to study molecular mechanisms of drug candidates

**Cyclin K/Cdk12: from identification of cellular function to synthesis of its first inhibitor/
set up of clinical trials**

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



CycK binds Cdk12



CycK /Cdk12 is necessary for expression of DNA damage response genes

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

Dalibor Blazek,^{1,2,9,10} Jiri Kohoutek,^{3,9} Koen Bartholomeeusen,¹ Eric Johansen,⁴ Petra Hulinkova,³ Zeping Luo,¹ Peter Cimermanic,^{5,6,7} Jernej Ule,⁸ and B. Matija Peterlin^{1,9}

¹Department of Medicine, Microbiology, and Immunology, Rosalind Russell Medical Research Center, University of California at San Francisco (UCSF), San Francisco, California 94143, USA; ²Central European Institute of Technology, Masaryk University, 62500 Brno, Czech Republic; ³Department of Toxicology, Pharmacology, and Immunotherapy, Veterinary Research Institute, 62100 Brno, Czech Republic; ⁴UCSF Sandler-Moore Mass Spectrometry Core Facility, UCSF Helen Diller Family Comprehensive Cancer Center, University of California at San Francisco (UCSF), San Francisco, California 94143, USA; ⁵Department of Bioengineering and Therapeutic Sciences, University of California at San Francisco, San Francisco, California 94158, USA; ⁶Department of Pharmaceutical Chemistry, University of California at San Francisco, San Francisco, California 94158, USA; ⁷California Institute for Quantitative Biosciences, University of California at San Francisco, San Francisco, California 94158, USA; ⁸MRC, Laboratory of Molecular Biology, Cambridge CB20QH, United Kingdom

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
<i>P53</i>	302 (96%)	63852	tumor suppressor
<i>BRCA1</i>	11 (3%)	9231	tumor suppressor
<i>NF1</i>	13 (4%)	3064	tumor suppressor
<i>CDK12</i>	9 (3%)	27	?
<i>BRCA2</i>	10 (3%)	5793	tumor suppressor
<i>RB1</i>	6 (2%)	2050	tumor suppressor

Molecular characterization of CDK12 mutations in ovarian cancer

Published online 20 February 2015

*Nucleic Acids Research, 2015, Vol. 43, No. 5 2575–2589
doi: 10.1093/nar/gkv101*

Ovarian carcinoma *CDK12* mutations misregulate expression of DNA repair genes via deficient formation and function of the Cdk12/CycK complex

Kingsley M. Ekumi^{1,†}, Hana Paculova^{2,†}, Tina Lenasi^{1,‡}, Vendula Pospichalova^{3,‡}, Christian A. Böskén⁴, Jana Rybarikova², Vitezslav Bryja^{3,5}, Matthias Geyer⁴, Dalibor Blazek^{2,*} and Matjaz Barboric^{1,*}

¹Institute of Biomedicine, Biochemistry and Developmental Biology, University of Helsinki, Helsinki FIN-00014, Finland, ²Central European Institute of Technology (CEITEC), Masaryk University, 62500 Brno, Czech Republic, ³Institute of Experimental Biology, Faculty of Science, Masaryk University, 61137 Brno, Czech Republic, ⁴Center of Advanced European Studies and Research, Group Physical Biochemistry, 53175 Bonn, Germany and ⁵Institute of Biophysics, Academy of Sciences of the Czech Republic, 61265 Brno, Czech Republic

Identification of the first inhibitor of CDK12

ARTICLE

PUBLISHED ONLINE: 29 AUGUST 2016 | DOI: 10.1038/NCEMBIO.2166

nature
chemical biology

Covalent targeting of remote cysteine residues to develop CDK12 and CDK13 inhibitors

Tinghu Zhang^{1,2,11}, Nicholas Kwiatkowski^{1-3,11}, Calla M Olson^{1,2,11}, Sarah E Dixon-Clarke⁴, Brian J Abraham³, Ann K Greifenberg^{5,6}, Scott B Ficarro^{1,2,7}, Jonathan M Elkins⁴, Yanke Liang^{1,2}, Nancy M Hannett³, Theresa Manz^{1,8}, Mingfeng Hao^{1,2}, Bartłomiej Bartkowiak⁹, Arno L Greenleaf⁹, Jarrod A Marto^{1,2,7}, Matthias Geyer^{5,6}, Alex N Bullock⁴, Richard A Young^{3,10*} & Nathanael S Gray^{1,2*}

Clinical trials evaluating CDK12 status as a biomarker In various cancer

Table 1. Clinical trials evaluating CDK12 mutational status as a biomarker in various cancer types

Trial name	CDK biomarker	Therapeutic intervention	Objectives
BRCAAway: A Randomized phase II trial of abiraterone, olaparib, or abiraterone + olaparib in patients with metastatic castration-resistant prostate cancer with DNA-repair defects (NCT03012321)	Patients with mCRPC and mutations in noncanonical DNA repair genes including <i>CDK12</i>	Olaparib, abiraterone	Evaluate the objective PFS of abiraterone/prednisone, olaparib or the combination abiraterone/prednisone + olaparib in patients with mCRPC
TRITON2: A multicenter, open-label phase II study of rucaparib in patients with metastatic castration-resistant prostate cancer associated with homologous recombination deficiency (NCT02952534)	Patients with mCRPC and mutations in noncanonical DNA repair genes including <i>CDK12</i>	Rucaparib	Evaluate the ORR and PSA response in patients with mCRPC
IMPACT: Immunotherapy in patients with metastatic cancers and <i>CDK12</i> mutations (NCT03570619)	Patients with mCRPC or other cancers and <i>CDK12</i> loss-of-function mutations	Ipilimumab plus nivolumab	Evaluate the ORR and PSA response in patients with mCRPC
Nivolumab in biochemically recurrent dMMR prostate cancer (NCT04019964)	Patients with biochemically recurrent prostate cancer after prior local therapy and no radiographic evidence of metastasis and <i>CDK12</i> -inactivating mutations or dMMR	Nivolumab	Evaluate the PSA50 response as well as the PSA PFS, metastasis-free survival, and time to initiation of next systemic therapy

Clinical trials evaluating CDK12 status as a biomarker In various cancer-cont.

Phase II trial of PARP inhibitor niraparib for men with high-risk prostate cancer and DNA damage response defects (NCT04030559)	Patients with high-risk localized prostate cancer and mutations in canonical and noncanonical DNA repair genes including <i>CDK12</i>	Niraparib	Evaluate the tumor stage, lymph node metastasis, margins, and pathologic CR rate at prostatectomy and PSA PFS
Combination therapy of rucaparib and irinotecan in cancers with mutations in DNA repair (NCT03318445)	Patients with advanced cancer and mutations in canonical and noncanonical DNA repair genes including <i>CDK12</i>	Rucaparib plus irinotecan	Evaluate the ORR as defined by the proportion of patients with either confirmed CR or partial response (as per RECIST)
ORCHID: Phase II study of olaparib in patients with metastatic renal cell carcinoma harboring a BAP1 or other DNA repair gene mutations (NCT03786796)	Patients with renal cell carcinoma and mutations in DNA repair genes including <i>CDK12</i>	Olaparib	Evaluate the ORR as defined by the proportion of patients with either confirmed CR or partial response (as per RECIST)
A phase Ib biomarker-driven combination trial of copanlisib, olaparib, and MEDI4736 (durvalumab) in patients with advanced solid tumors (NCT03842228)	Patients with advanced metastatic cancer and germline or somatic mutations in DNA damage repair genes, including <i>CDK12</i>	Copanlisib, olaparib, plus durvalumab	Evaluate the MTD of copanlisib and olaparib. Secondary objectives include assessment of the ORR as defined by the proportion of patients with either confirmed CR or partial response (as per RECIST)

Currently several biotech companies develop CDK12 inhibitors for clinical trials