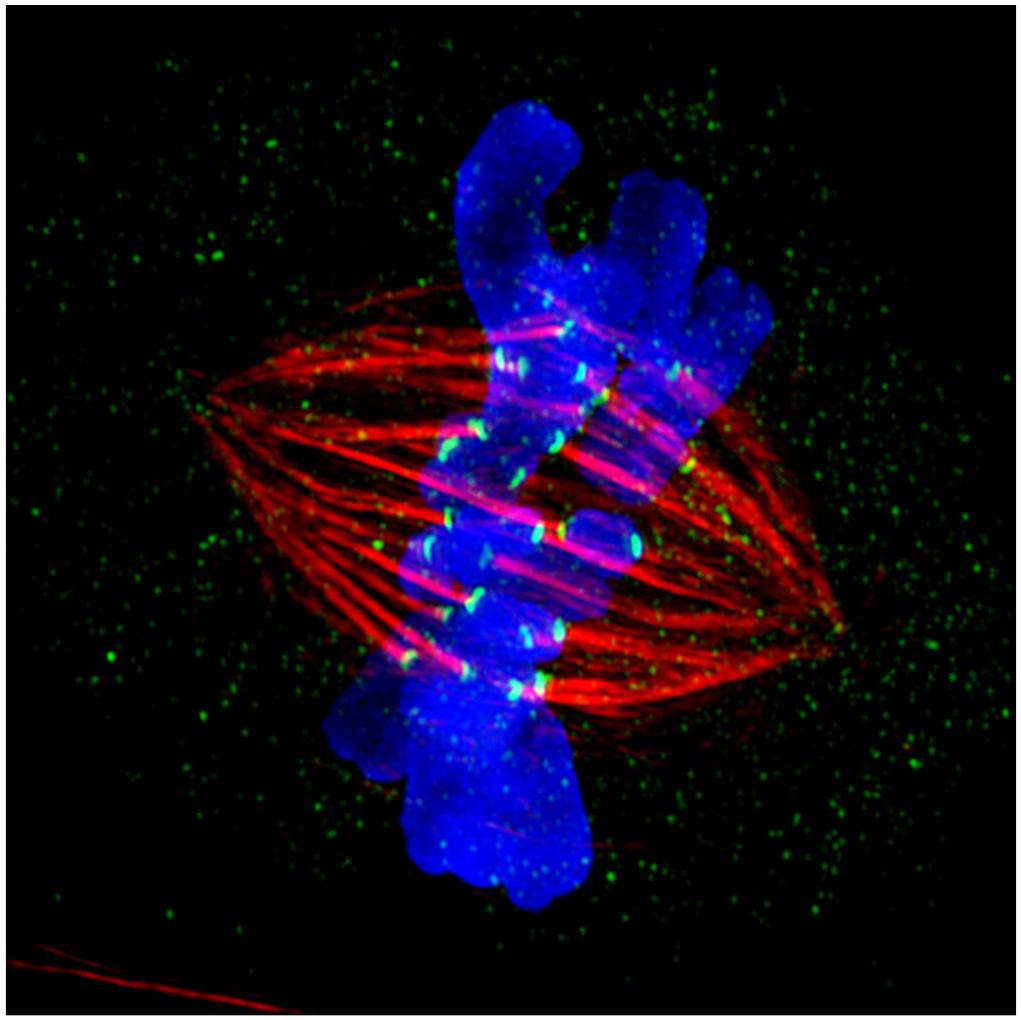
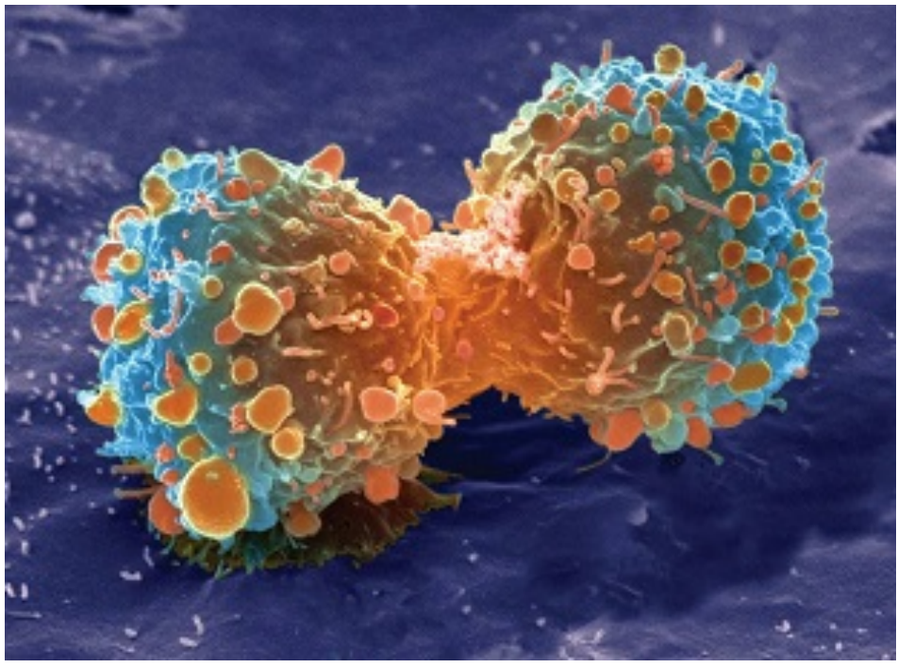
# *Targeting Cell Cycle Proteins for Cancer Therapeutics*



**Group Meeting**

November 3, 2021

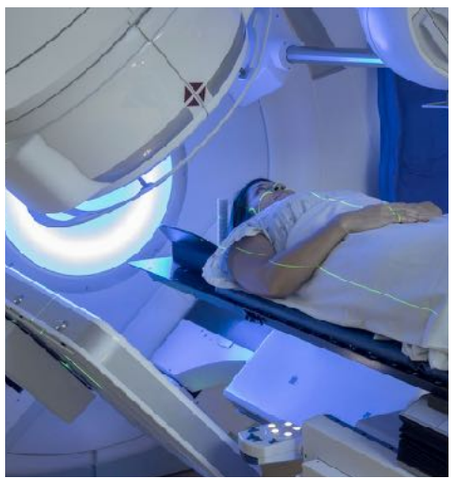
# *Cancer*



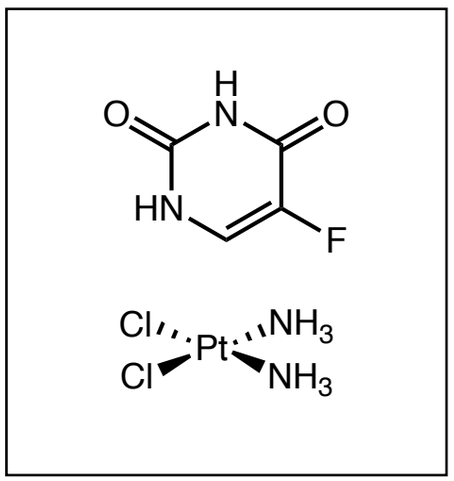
**Family of diseases** arising from uncontrolled division of abnormal cells

- Second leading cause of death globally (1 in 6)
- 90 million newly reported cases annually
- Global economic burden exceeds $2 trillion USD

*surgery*



*radiotherapy*




*chemotherapy*

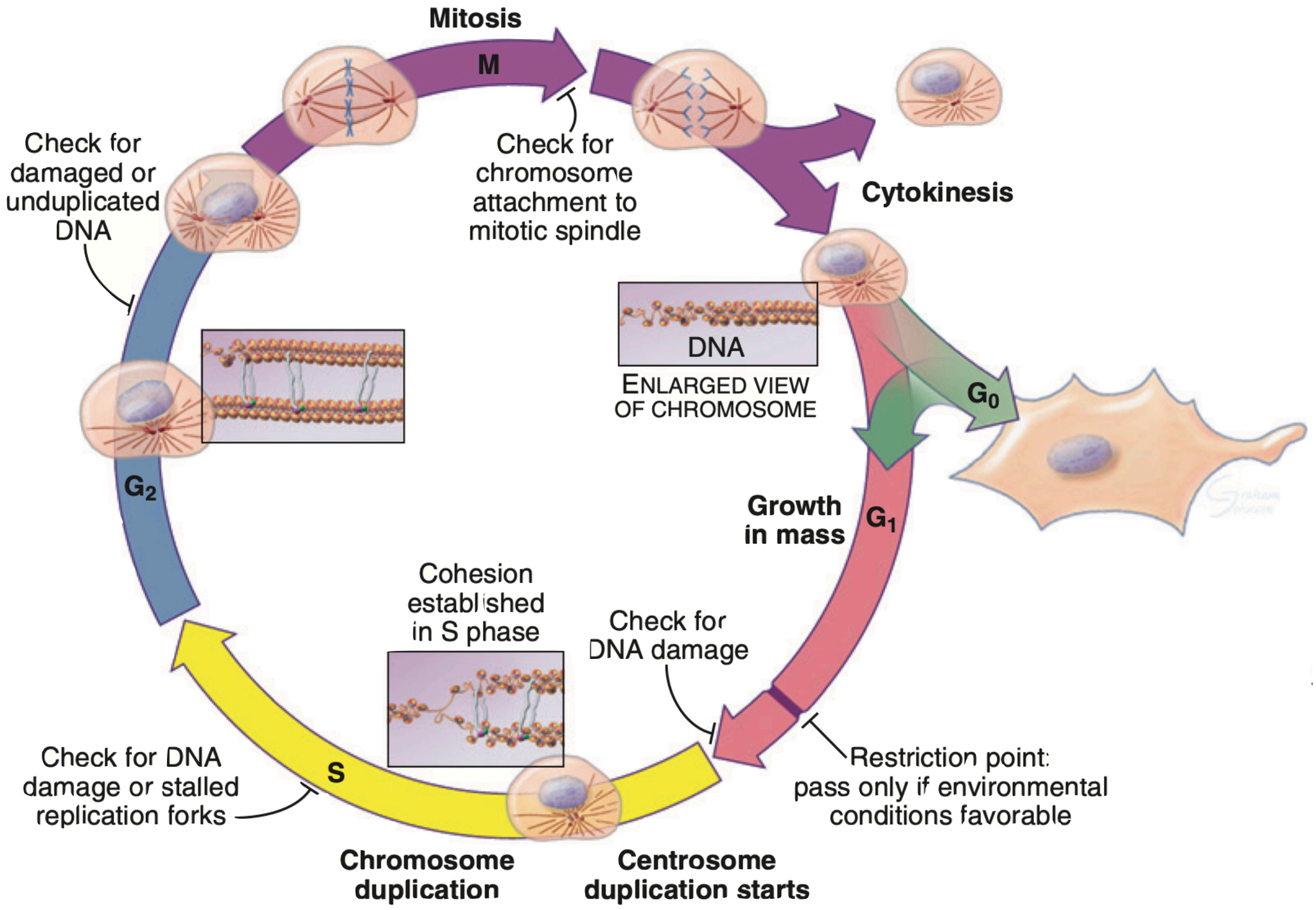
**targeted therapies** selectively kill certain types of cancer cells in patients with relevant **biomarkers**

Sudhakar, A. *J. Cancer Sci. Ther.* **2009**, *1,* 1.

# *Outline*

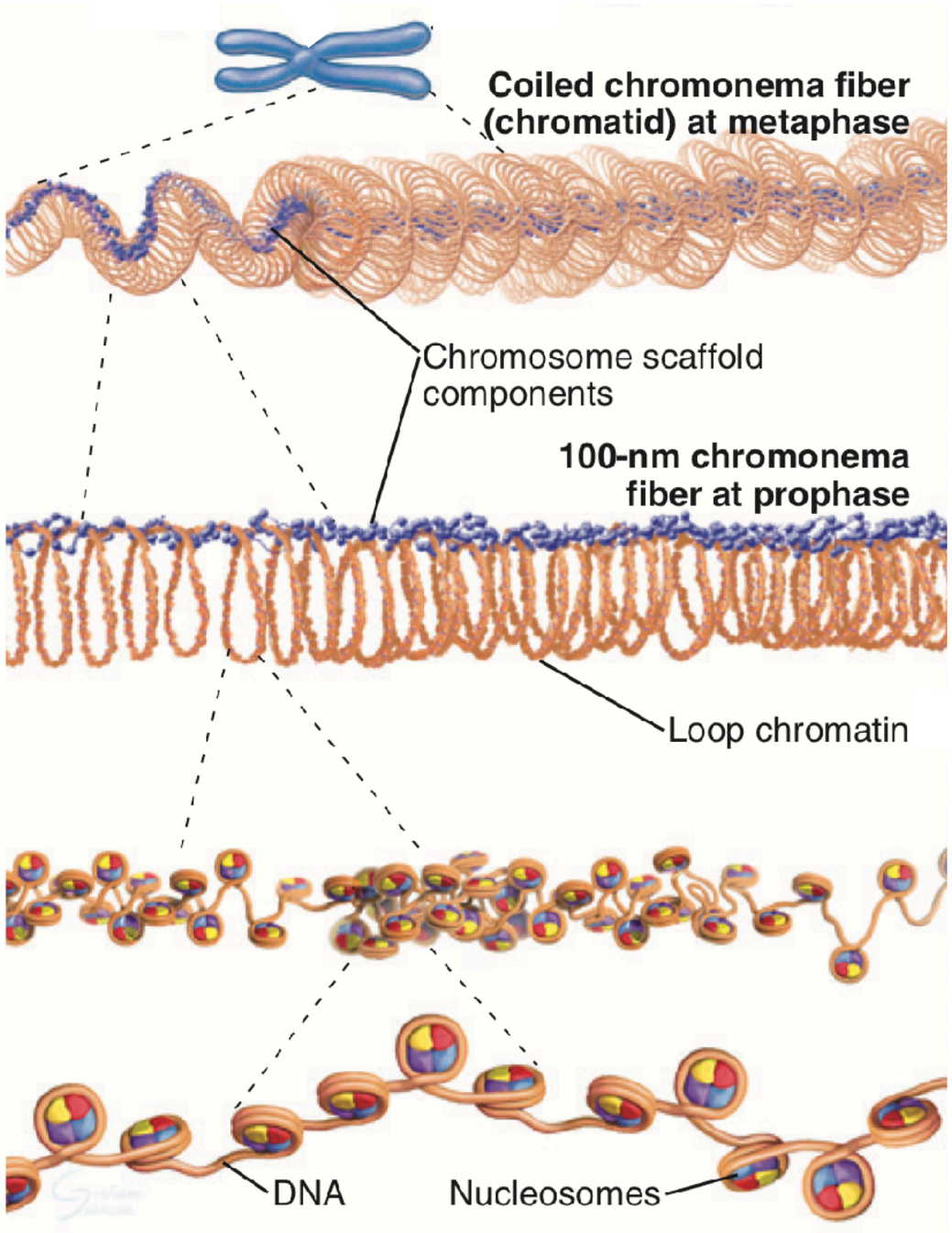
- Part I: Introduction to the Cell Cycle and Basic Concepts
- Part II: Biochemical Regulation of Cell Cycle Progression
- Part III: Strategies for Therapeutic Intervention

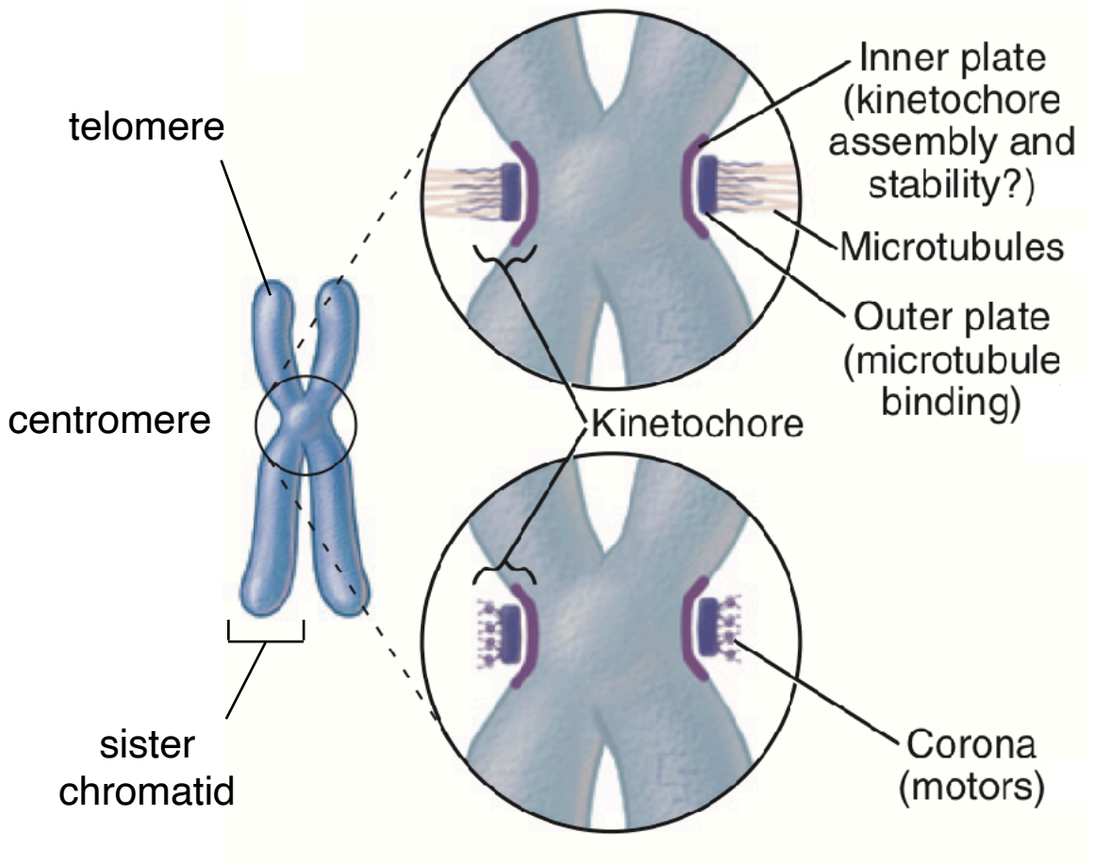
# *Overview of the Cell Cycle*

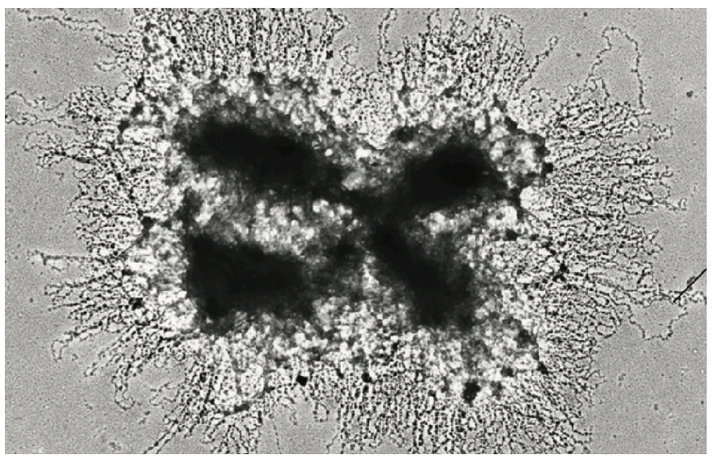



Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

# *DNA is Packaged into Chromosomes and Chromatin*

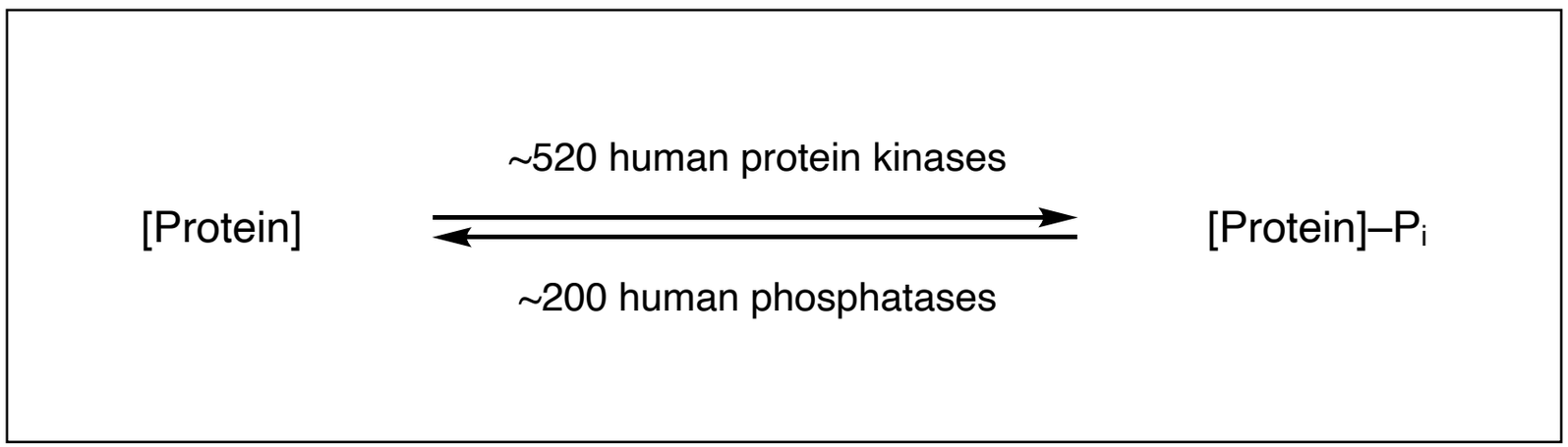


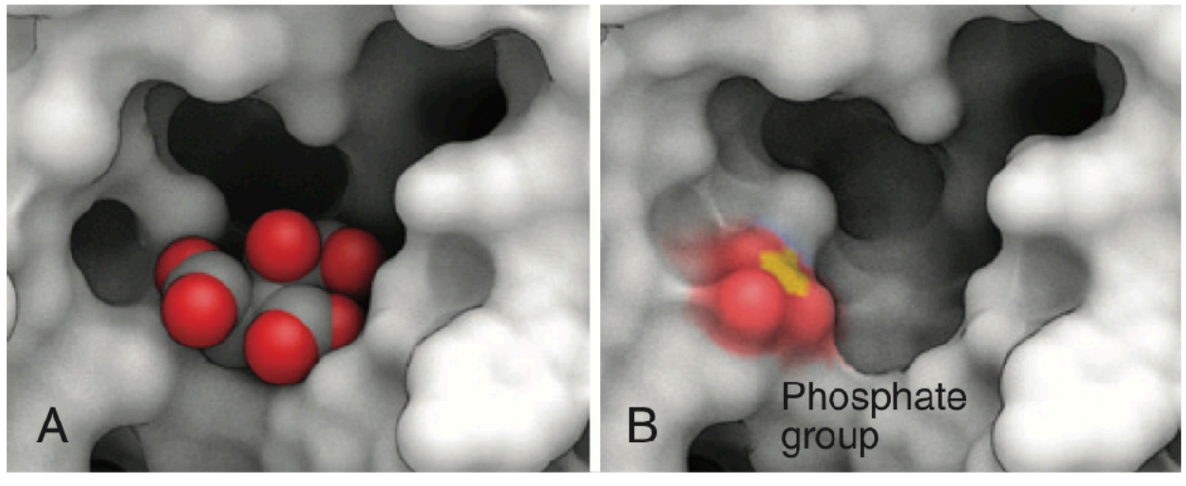



Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

# *Kinases and Phosphatases Control Signaling via Phosphorylation State*




inhibitory S113 phosphorylation of isocitrate dehydrogenase




**effects of phosphorylation**

---

direct interference

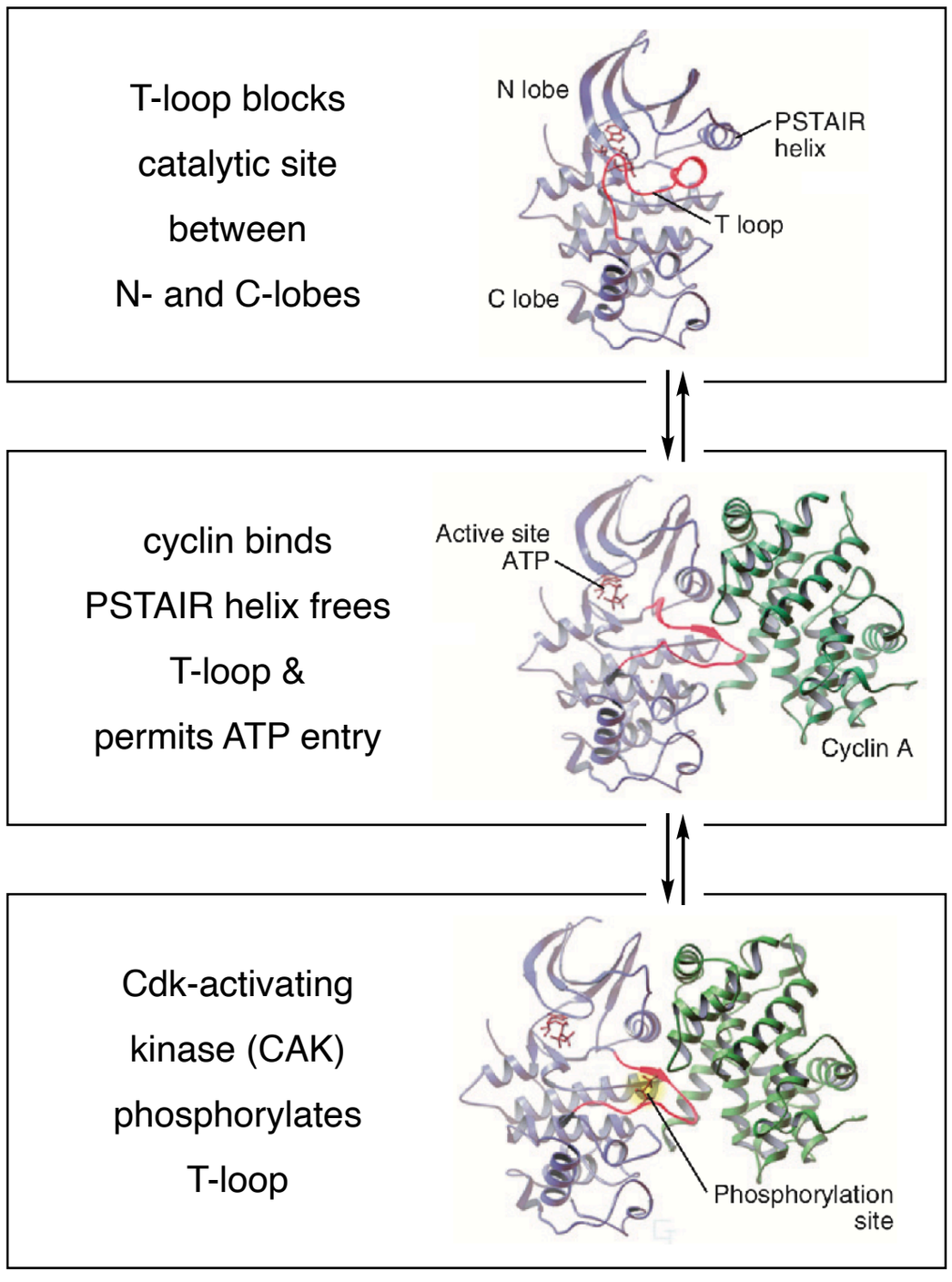
conformational change

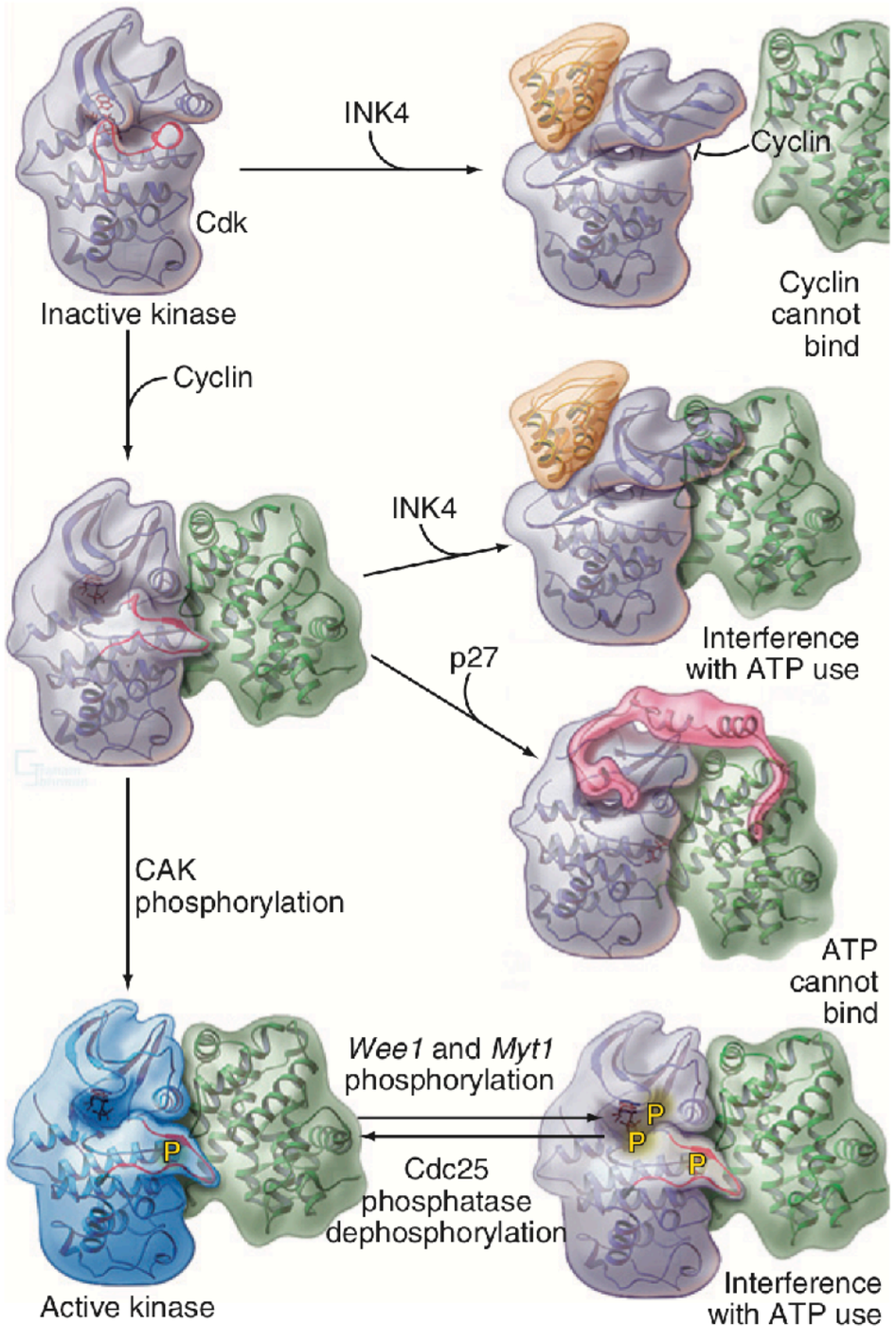
creation of binding sites

***kinases can be inhibited or activated themselves by phosphorylation***

Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

# *Cyclin-Dependent Kinases (CDKs) Drive Cell-Cycle Transitions*




Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

# *Ubiquitylation Targets Proteins to the 26S Proteasome for Destruction*

**Activation of ubiquitin by E1**

E1-enzyme conjugates C-terminal carboxyl of ubiquitin onto itself

**Transfer to E2**

Activated ubiquitin is transferred to E2 carrier enzyme at cysteine

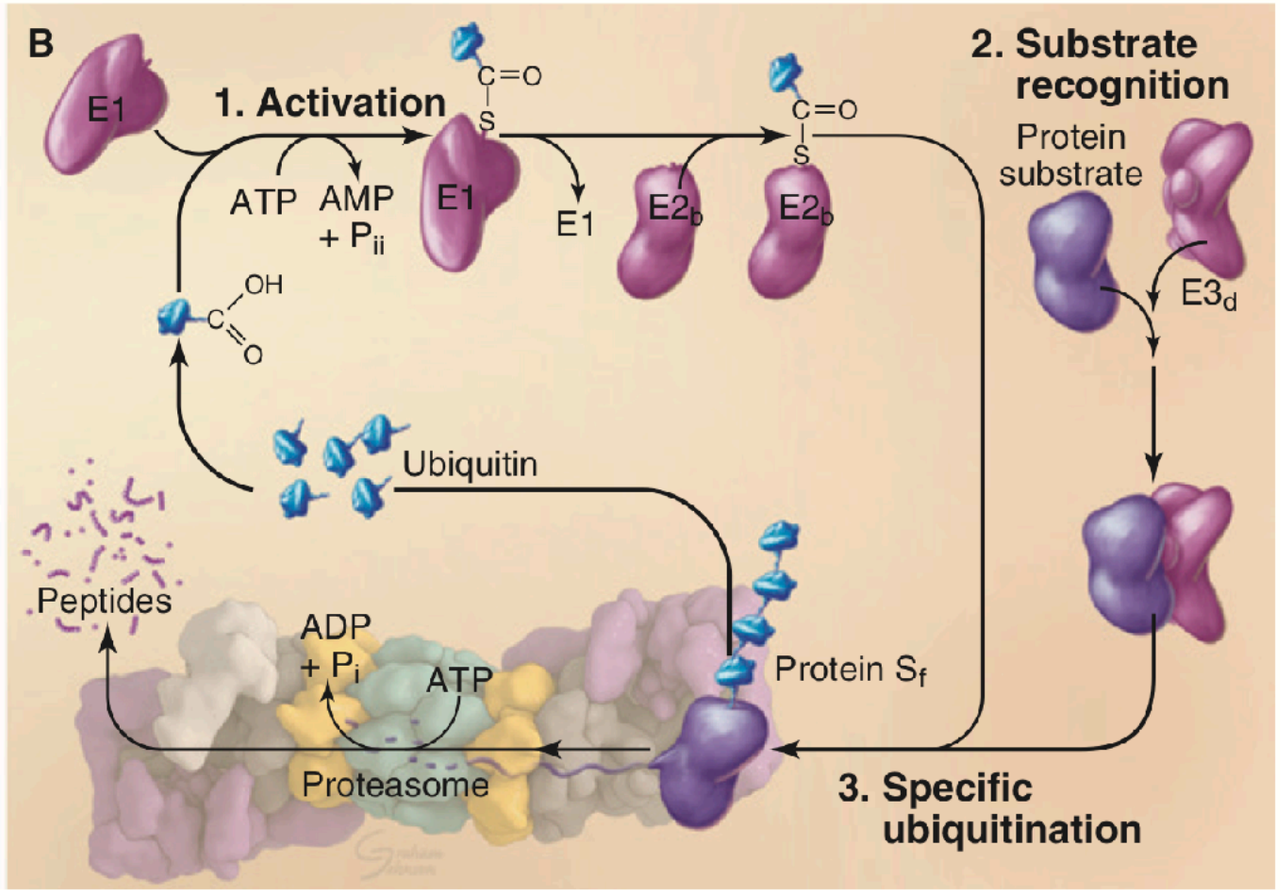
**Ubiquitylation of Target**

E3 ligases facilitate transfer either directly or via E3 intermediate

**Poly-ubiquitylation**

Poly-ubiquitin chain recognized by proteasome receptor machinery

2 **E1 enzymes** → 35 **E2 enzymes** → >600 **E3 enzymes**




**Other outcomes**: directs protein sorting, protein protein interactions, removal by deubiquitinases

Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

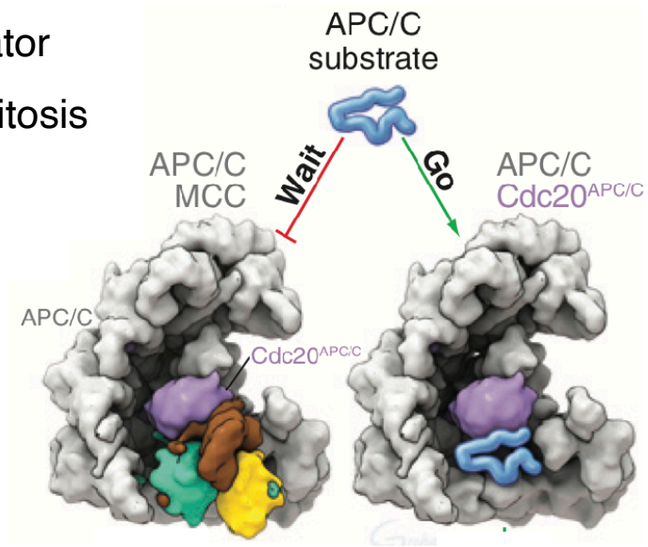
# *E3 Ligases APC/C and SCF Control Protein Degradation in the Cell Cycle*

**Anaphase-promoting complex/cyclosome**

Co-activators target complex to specific **degrons**

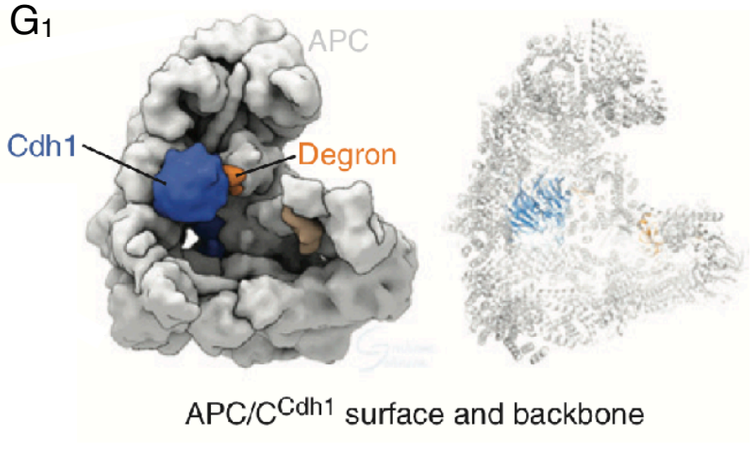
**Cdc20** coactivator

active during mitosis



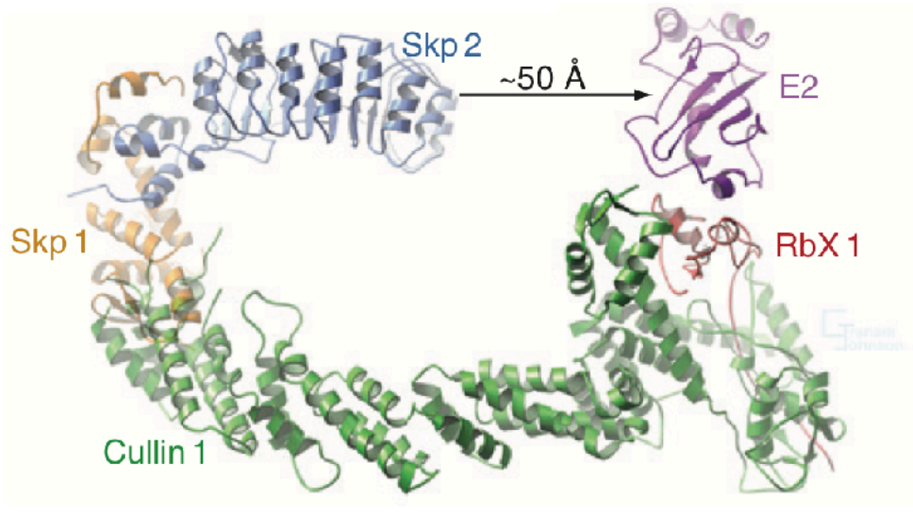

**Cdh** coactivator

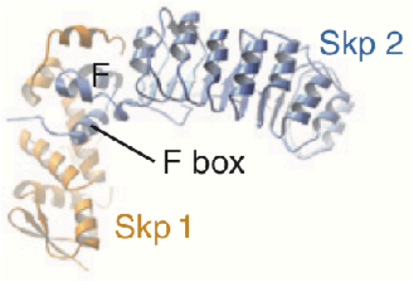
active during $G_1$




**Skp/cullin/F-box complex** is active during $S/G_2$

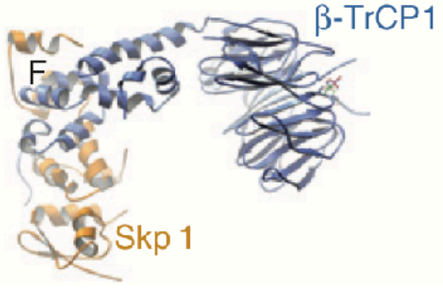
Specificity controlled by 78 **F-box proteins**




E2F-1: Cell-cycle regulator

$p27^{Kip1}$: Cdk2 inhibitor




Cdc25A: Cdk1 activator

Wee1: Cdk1 inhibitor

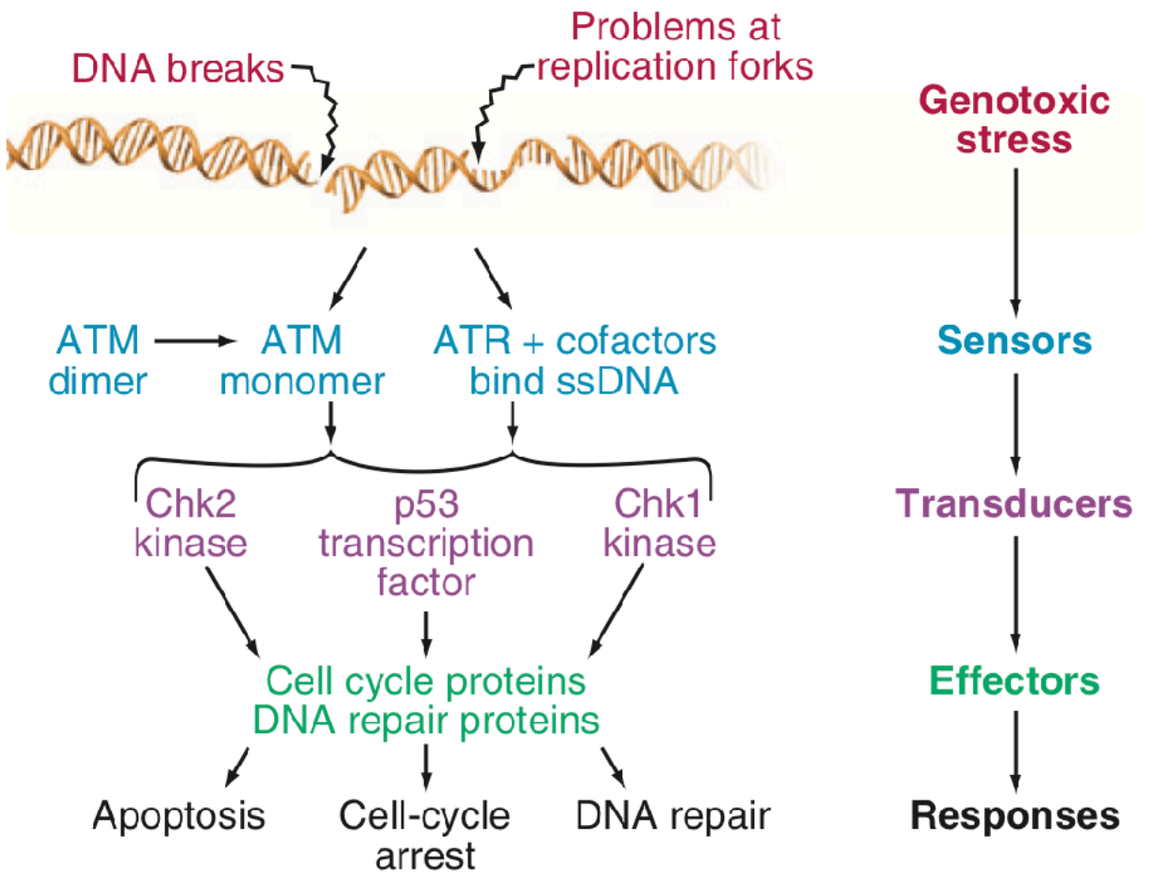
Emi1: $APC/C^{Cdh1}$ inhibitor

β-Catenin: Cell-proliferation regulator

Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

# *DNA Surveillance Mechanisms Operate Throughout Interphase*

**DNA damage** and **DNA replication stress** checkpoints control cell cycle progression and DNA repair




**Genotoxic stress**

caused by chemical agents, UV light, normal metabolism, viral infection, etc.

**Sensors**

recognize & bind sites of damaged DNA

**Transducers**

produce and amplify biochemical signals in response to damage

**Effectors**

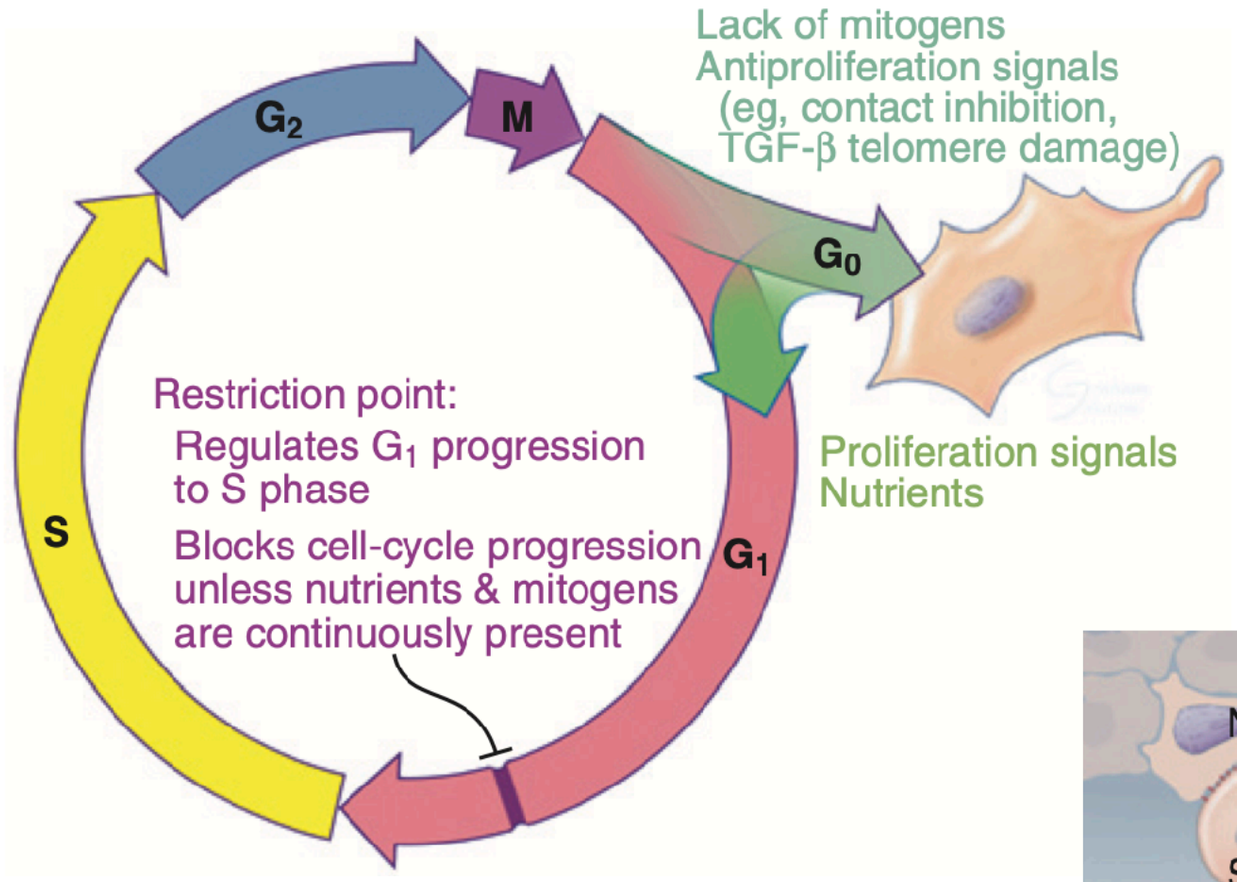
stall cell cycle and repair lesions

**Responses**

restrict propagation of damage

Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

# *$G_1$ Phase and Regulation of Cell Proliferation*




**Restriction Point**

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biochemical “gate” controlling progression

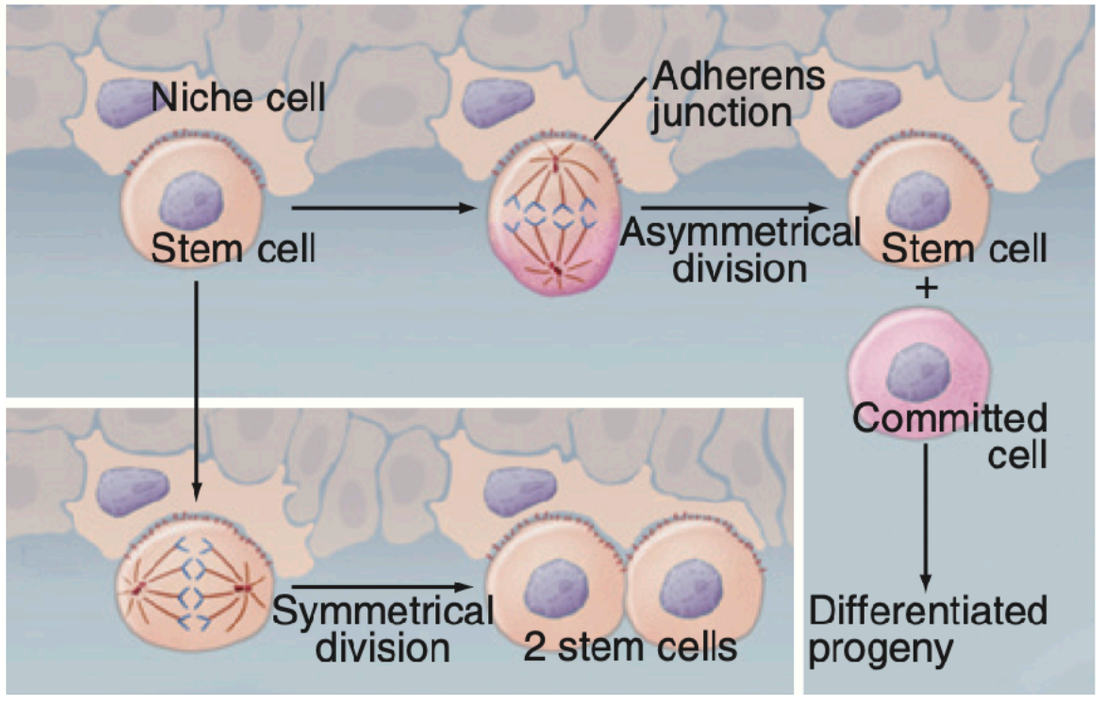
activates cell-cycle genes for transcription

**Cells in $G_0$ states**

***quiescent*** (reversible)

vs.

***senescent*** and ***differentiated*** (irreversible)




Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

# *Restriction Point Passage is Controlled by Cdk–Cyclin Activity*

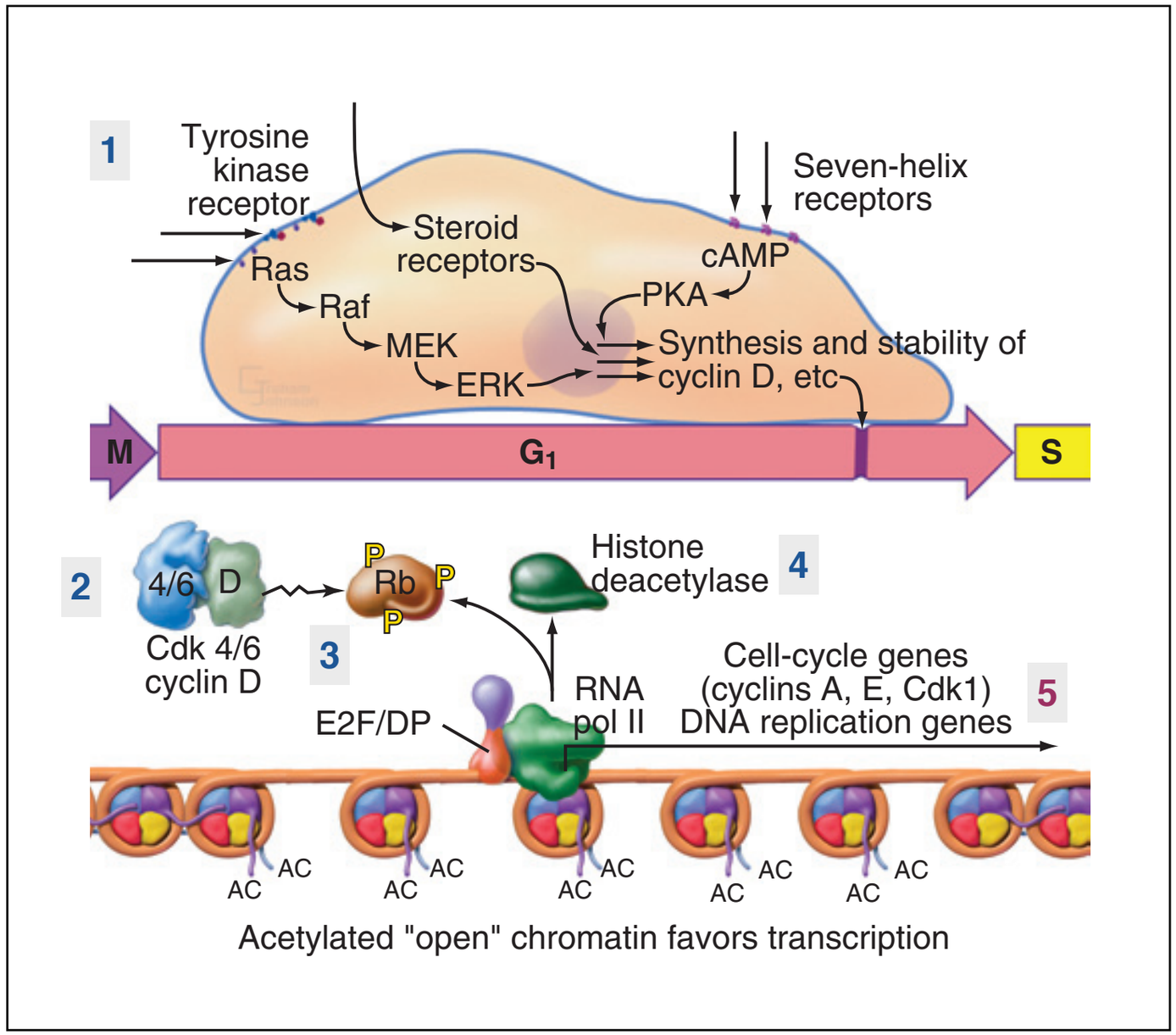
1. **Mitogens** stimulate expression of **cyclin D** via surface receptor transduction pathways

2. **Cyclin D** binds **Cdk4/6** and activates its kinase activity

3. **Cyclin D–Cdk4/6** phosphorylates **Rb,** releasing the **E2F** transcription factor

4. Rb dissocation permits histone acetylation and polymerase access

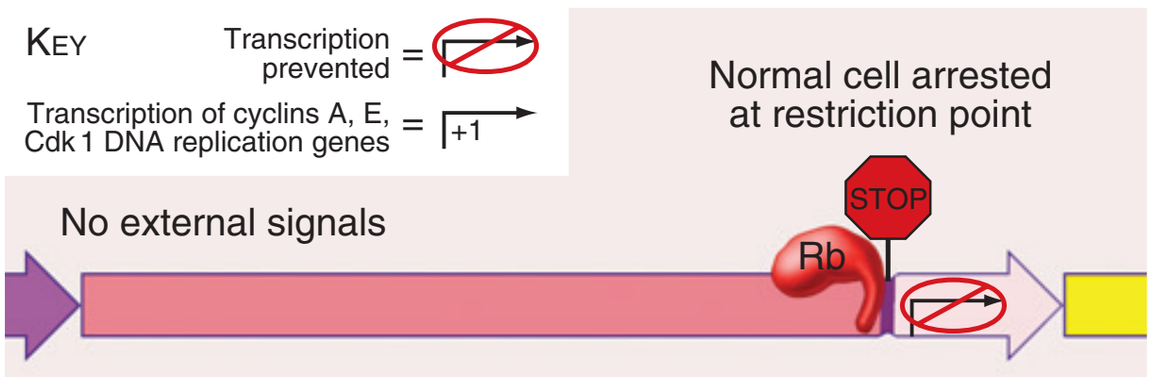
5. **E2F** activates transcription and expression of cell-cycle genes



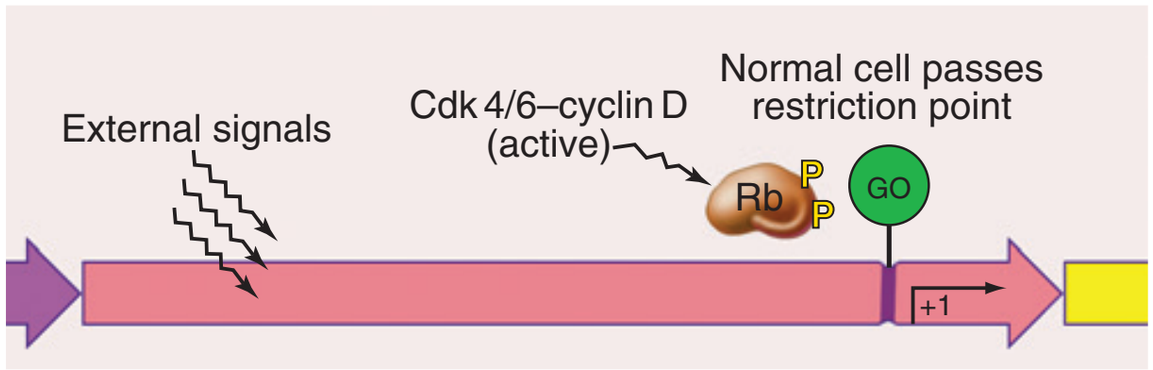

Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

# *Restriction Point Function and Cancer*

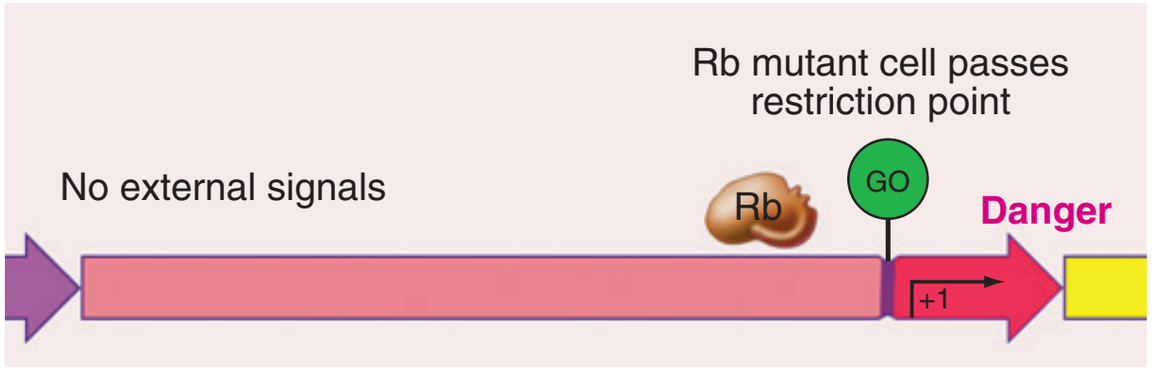
## normal $G_1$ stall

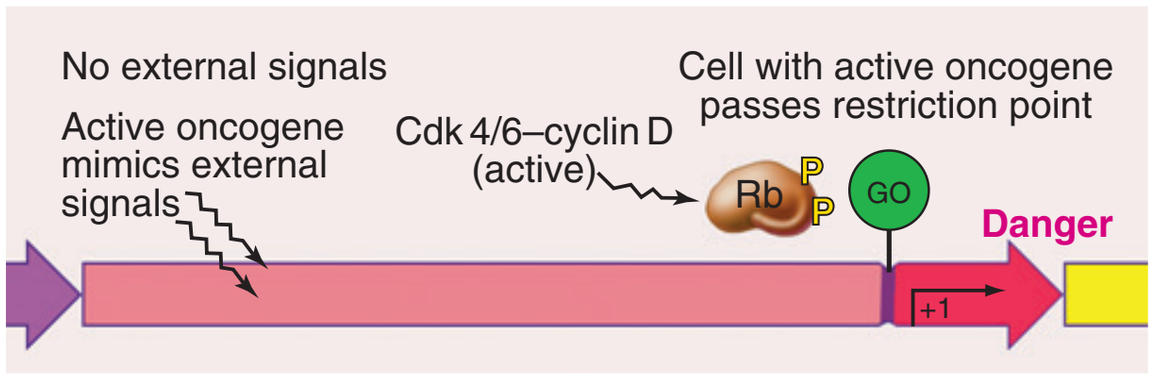



## normal $G_1$ progression




## oncogenic $G_1$ progression




mutations in the cyclin D–Cdk4/6–Rb–E2F pathway

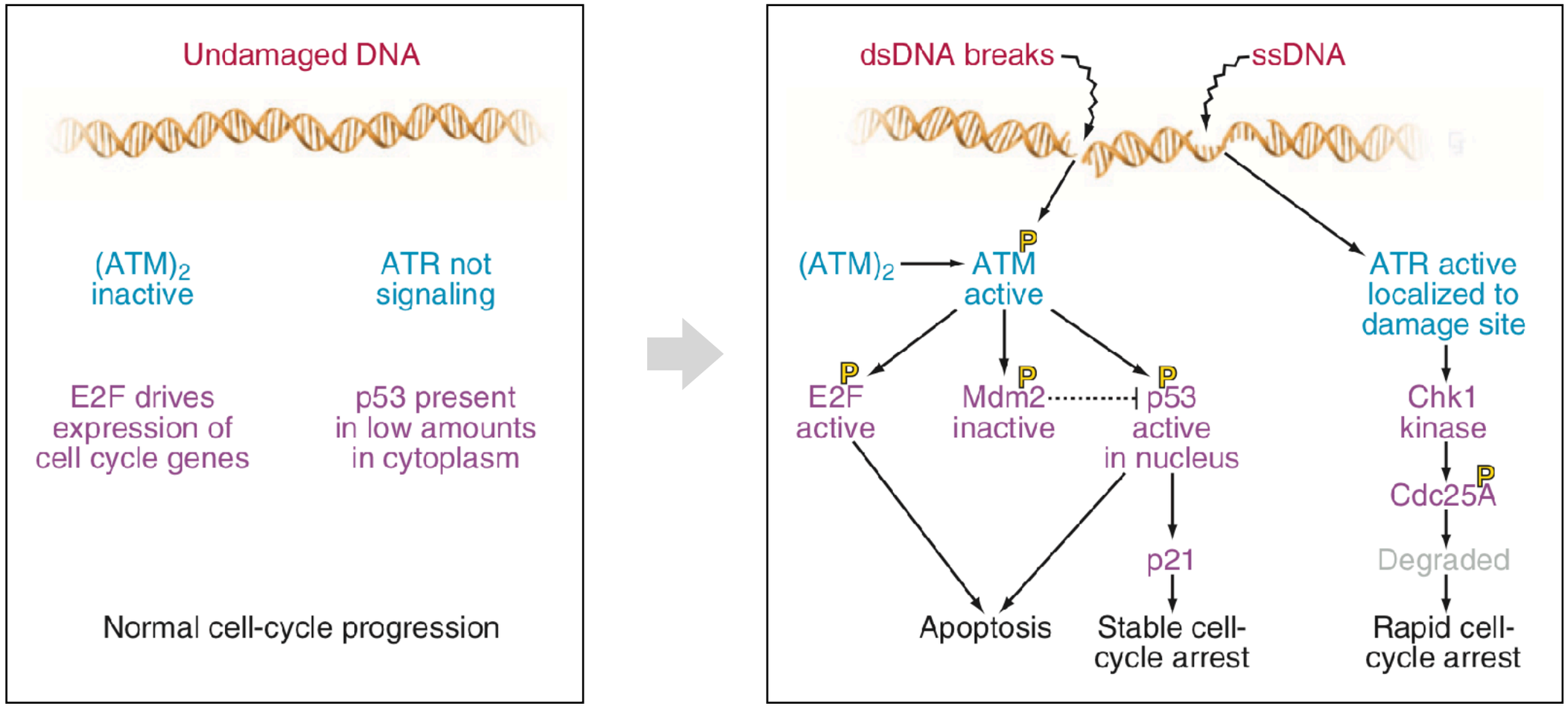
(or regulatory proteins) are common in cancers

active signaling by an oncogene (e.g., Ras)

tricks the cell into passing the restriction point

Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

# *$G_1$/S Checkpoint for DNA Damage: Two Pathways for Cell Cycle Arrest*




***p53 "guardian of the genome"***

**dsDNA breaks**

**ATM/p53** transcription factor activation: removing the wheels

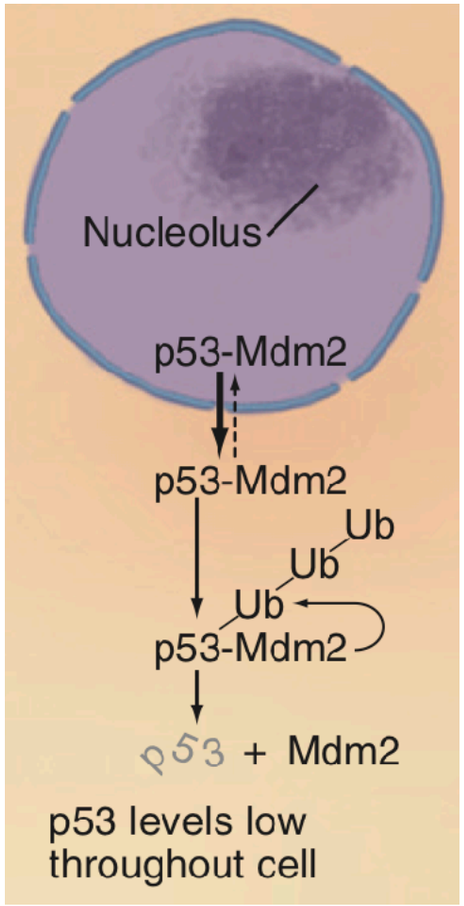
**exposed ssDNA**

**ATR/Chk1** kinase activation: applying the brakes

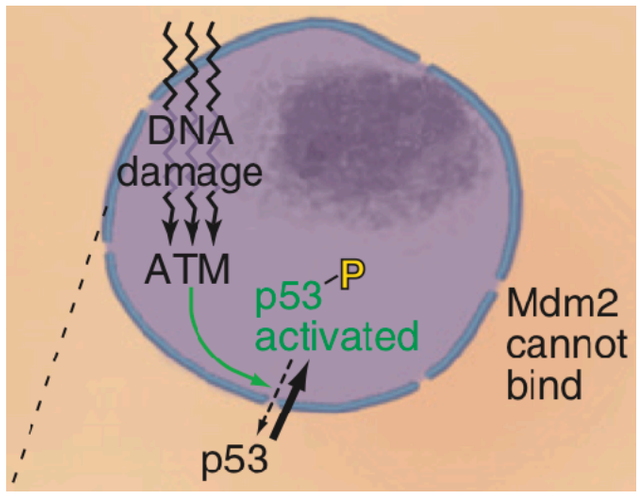
Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

# *p53 Regulation of the $G_1$/S Checkpoint for DNA Damage*

**healthy cell**



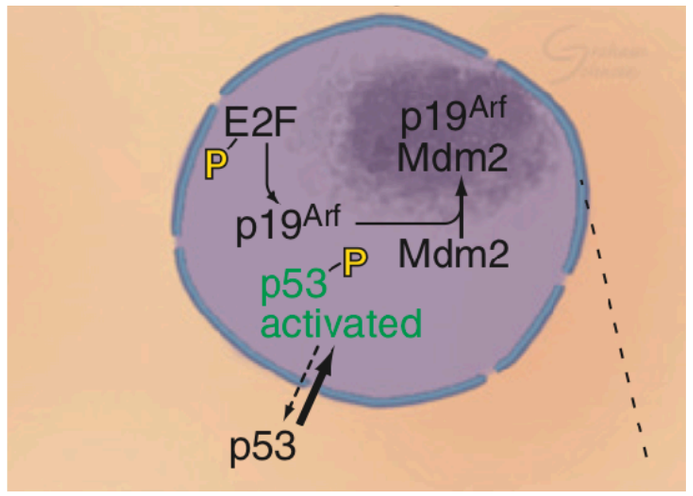

**irradiation**




ATM phosphorylates and activates p53

Mdm2 binding is blocked

**oncogenic stress**




E2F (from activated oncogene, e.g. K-Ras) transcribes p19

p19 sequesters Mdm2 in nucleolus

**p53** tumor suppressor

mutated/deleted in ~50% of human cancers

**Mdm2** oncogene

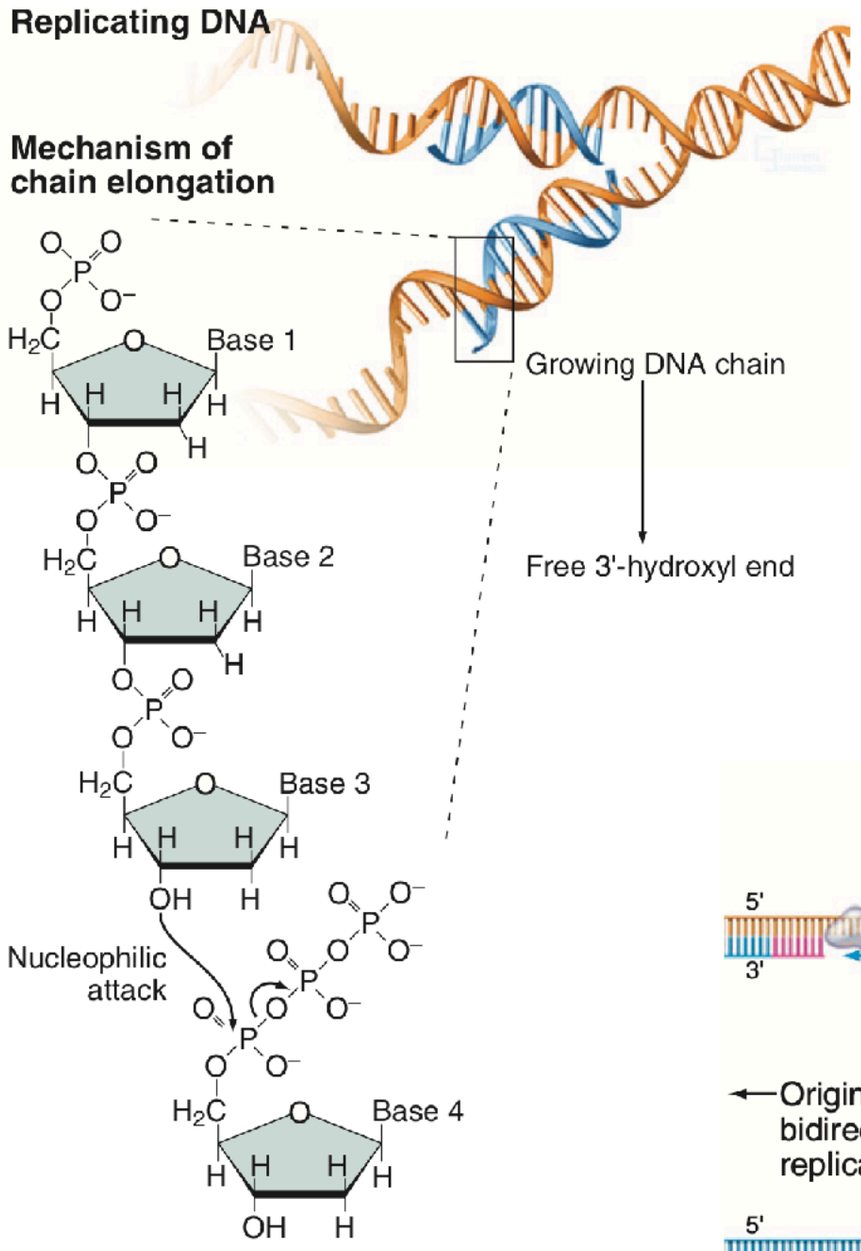
amplified/mutated in 57% of sarcomas

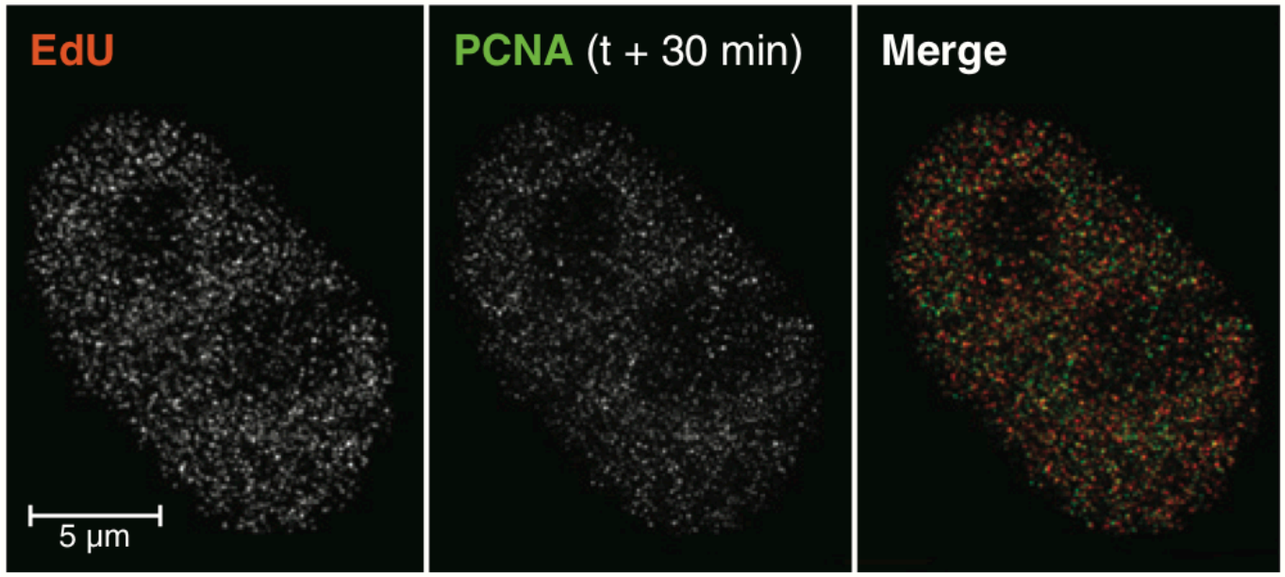
**p19** tumor supressor

mutated/deleted in up to 70% of human cancers

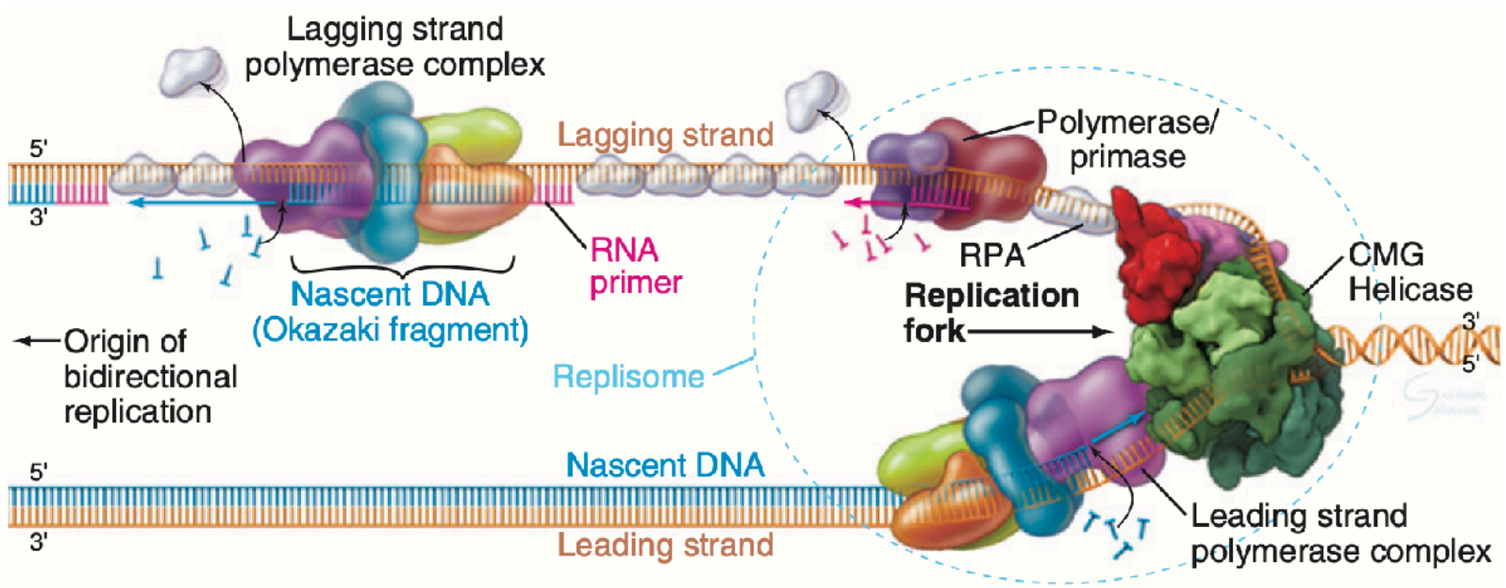
Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

# *S Phase and DNA Replication*




S-phase cell with thousands of replicons



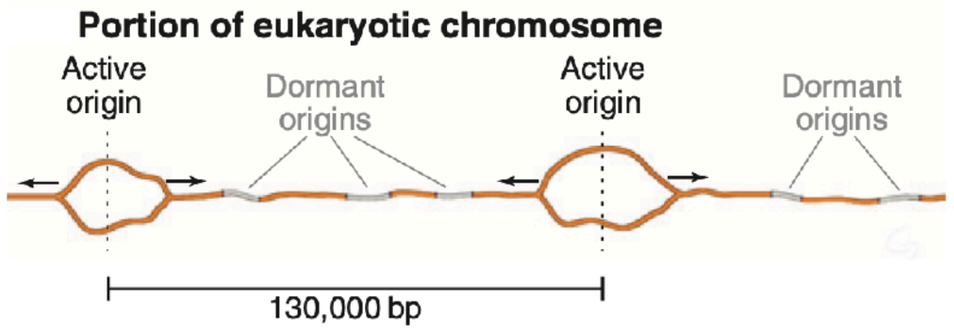

Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

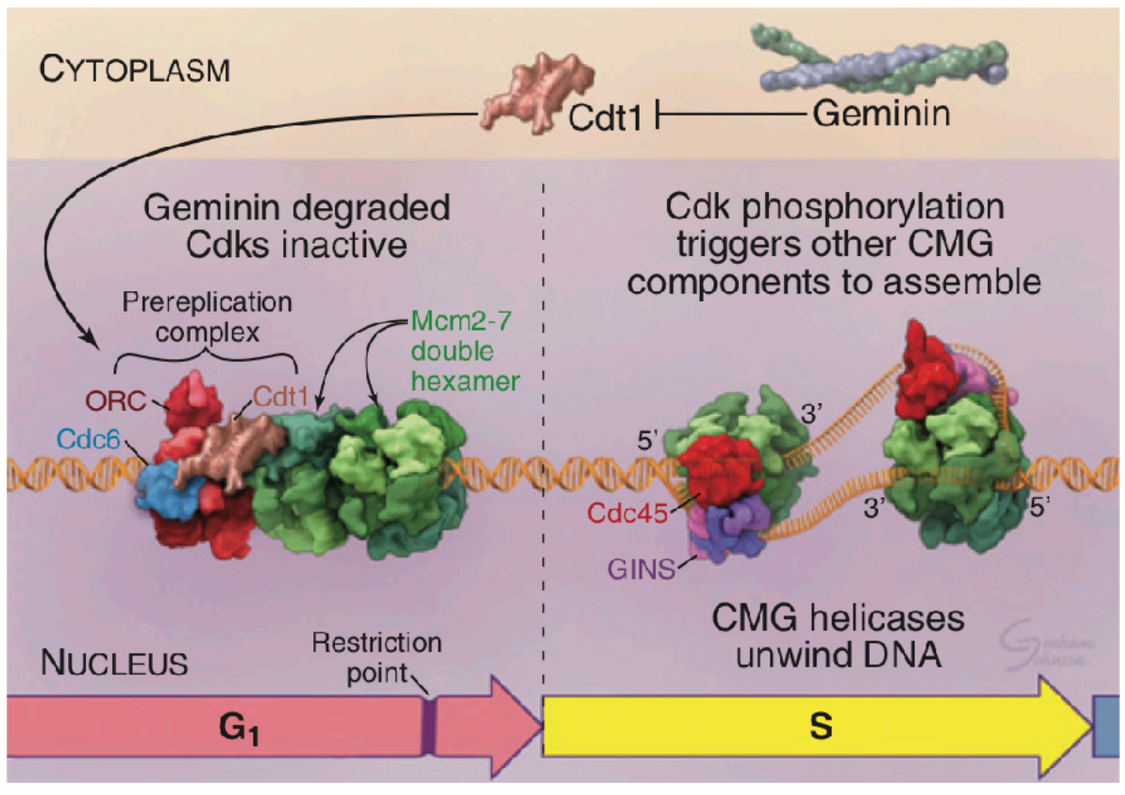
# *S Phase and DNA Replication*

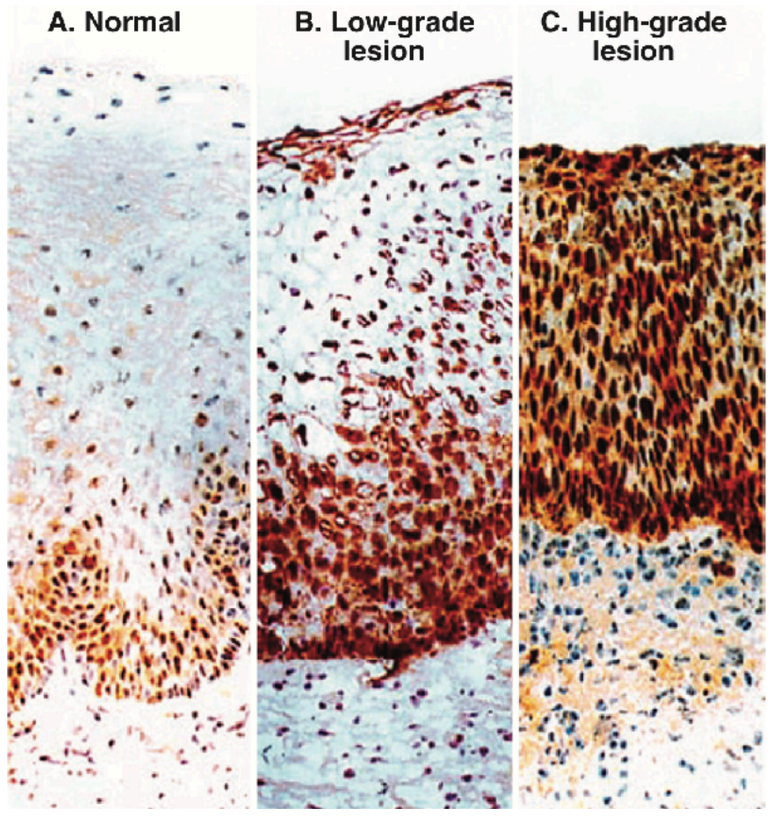
## Origin "Licensing" & "Firing"

origins are "licensed" in late $G_1$ (low Cdk-activity);

subset (10k) is "fired" during S-phase (high Cdk-activity)




**licensing** **firing**

human cervical epithelium (**anti-Mcm5**)

Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

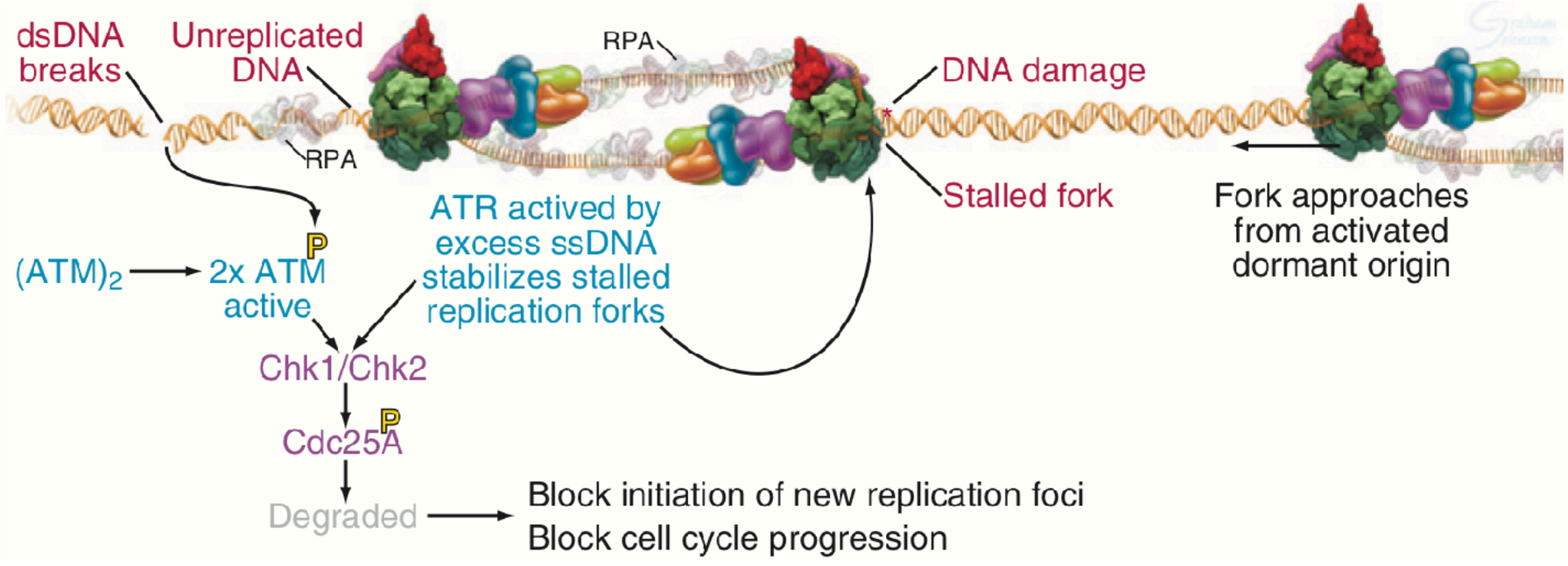
# *DNA Replication Stress Checkpoint*

***What happens if the replication fork hits an obstacle and stalls?***

e.g., bulky lesions, ribonucleotide, intra-strand H-bonding, etc.

**replication stress**

accumulation of ssDNA




**ATR activation** protects stalled forks from **replication fork collapse**

→ firing of nearby **dormant origin** leads to convergence at site of damage

→ small “gap” is traversed by a special **translesion DNA polymerase**

Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

# *$G_2$/Mitosis Transition Promoted by Cdk1 Feedback*

**molecular "switch"** flipped by **Polo kinase**

**positive feedback**

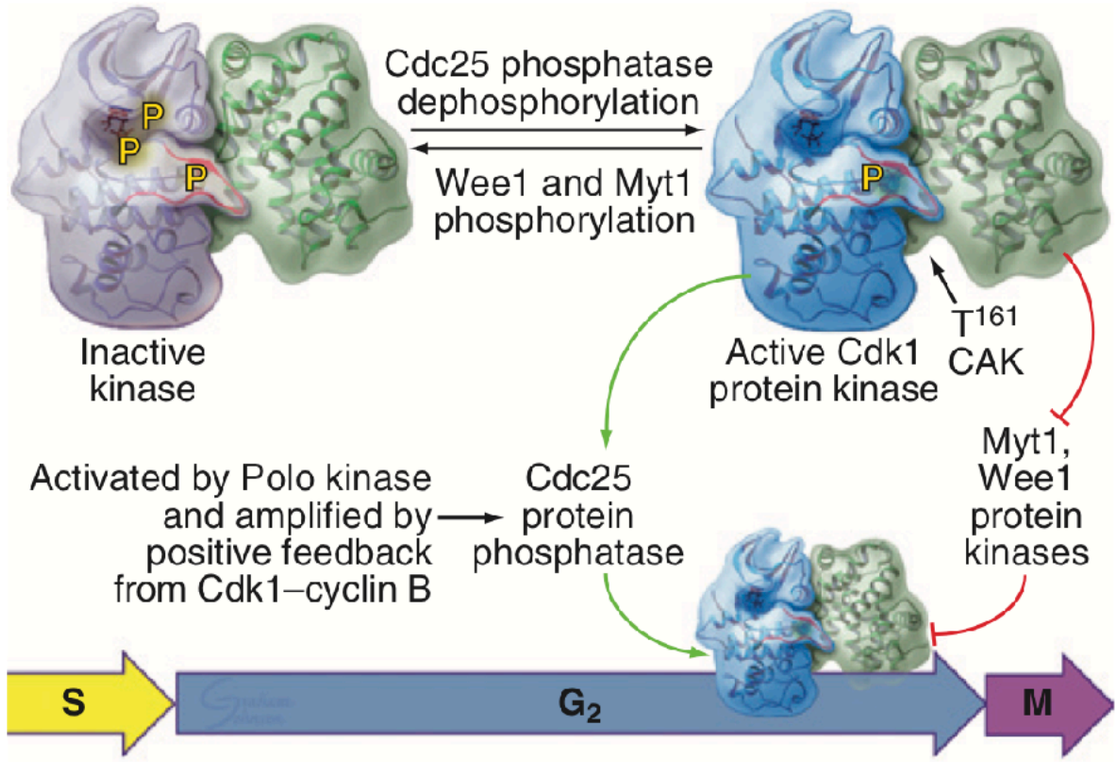
active Cdk1 phosphorylates and *activates*

its own activator phosphatase **Cdc25**

**inhibition of inhibition**

active Cdk1 phosphorylates and *inactivates*

its own inhibitory kinases **Wee1** and **Myt1**




**$G_2$/M transition events**

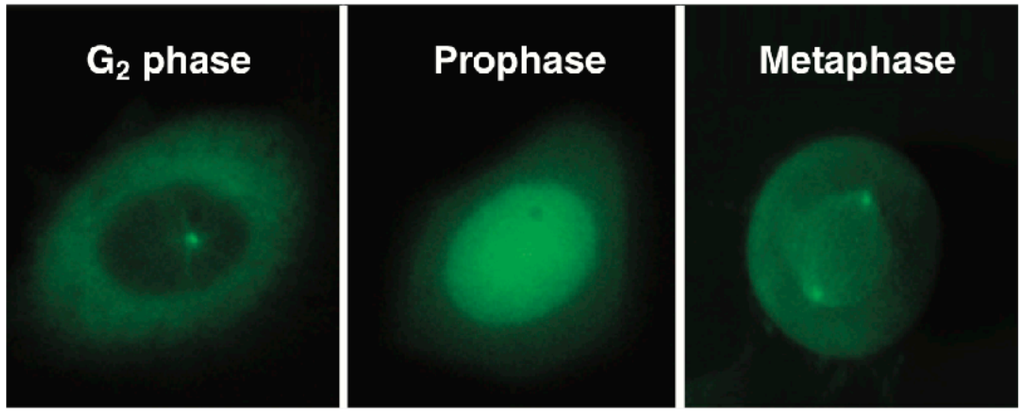
microtubule stability drops

centrosomes migrate apart

chromosomes condense

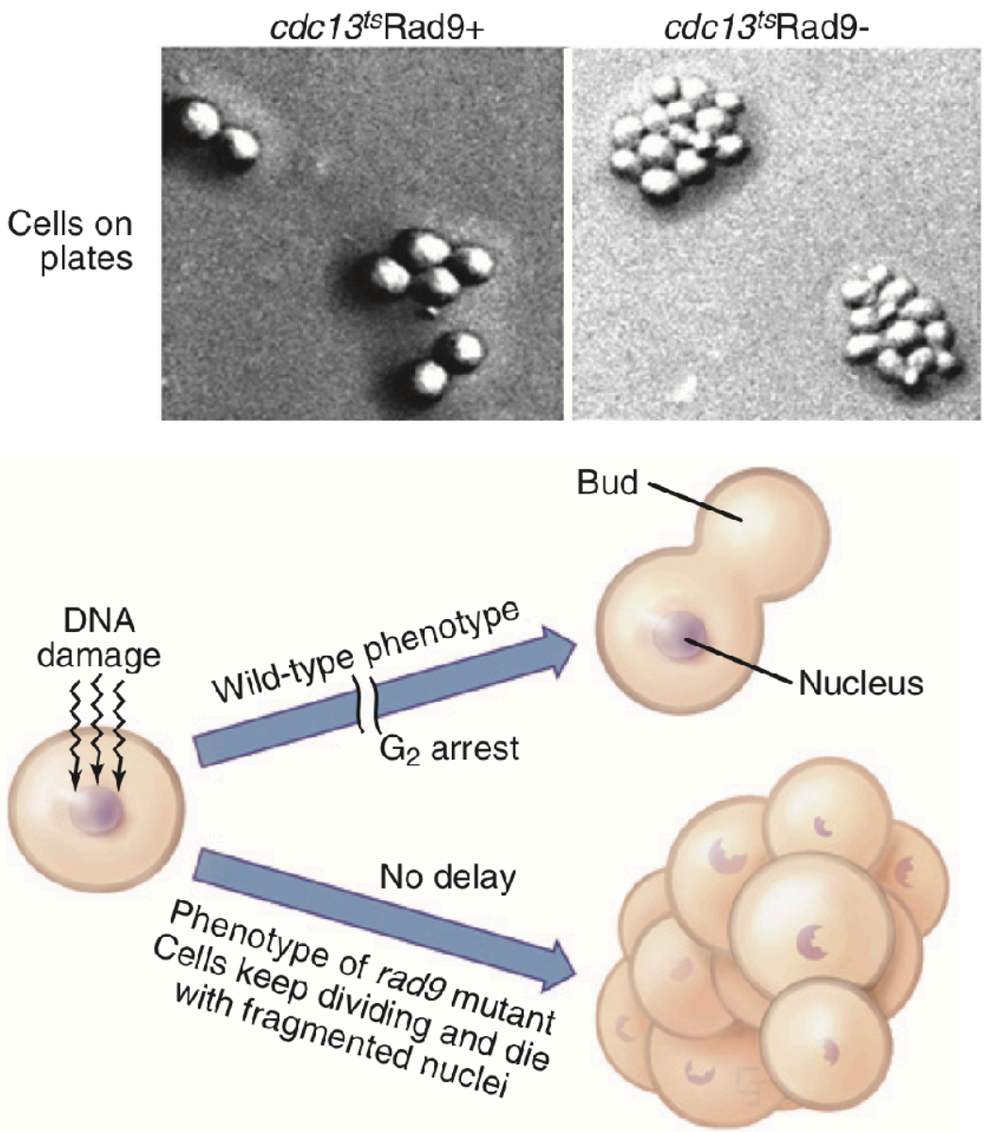
kinetochore assembly starts

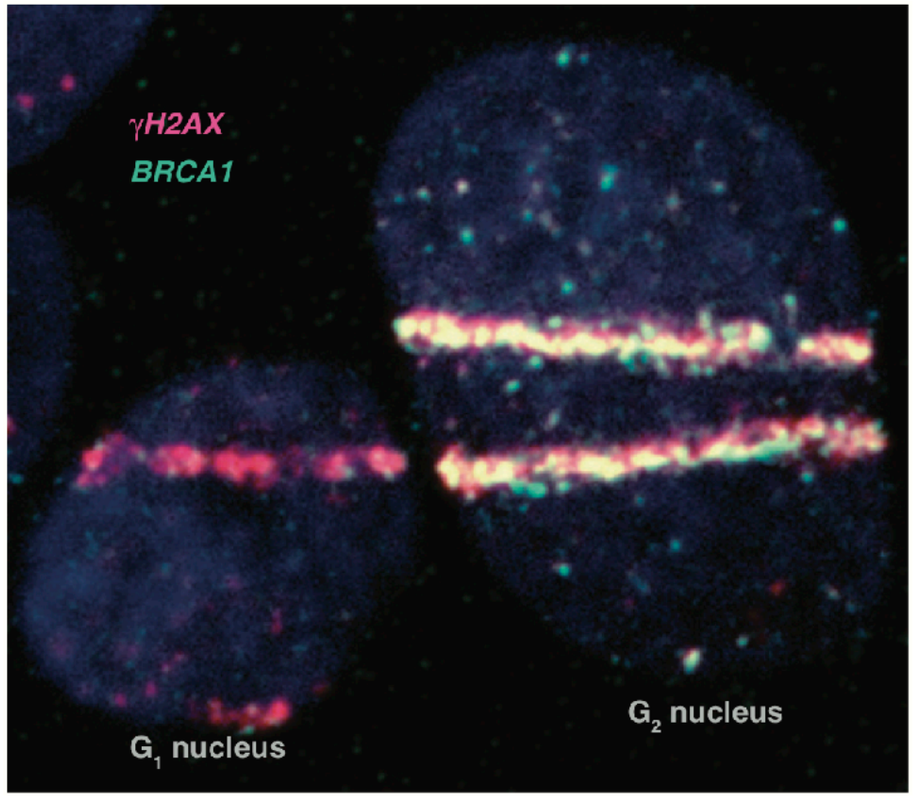
**anti-cyclin B1**




Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

# $G_2$ DNA Damage Checkpoint




**common DNA repair pathways in vertebrates**

base excision repair

nucleotide excision repair

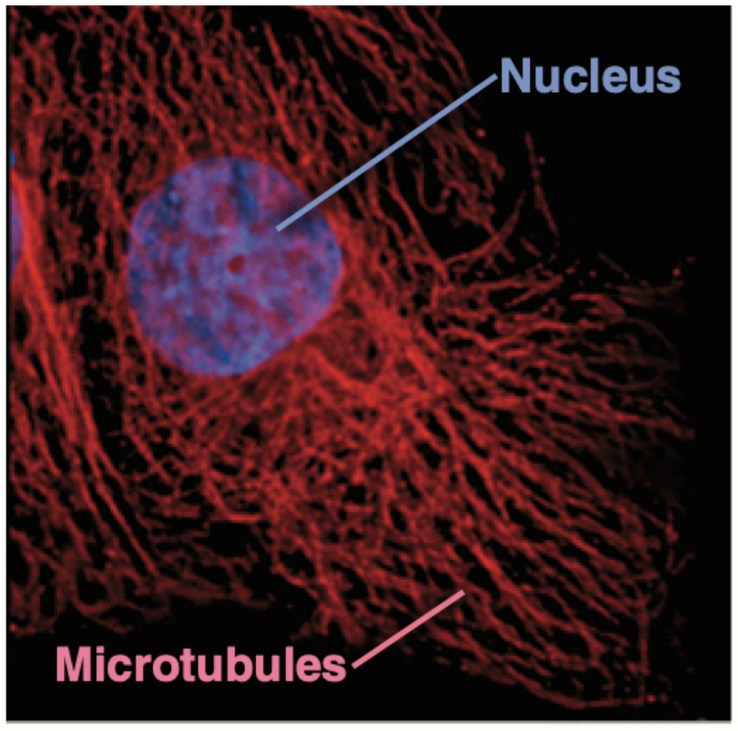
mismatch repair

double-stranded break repair (NHEJ or HR)

Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

# *Morphological Changes During Mitosis*

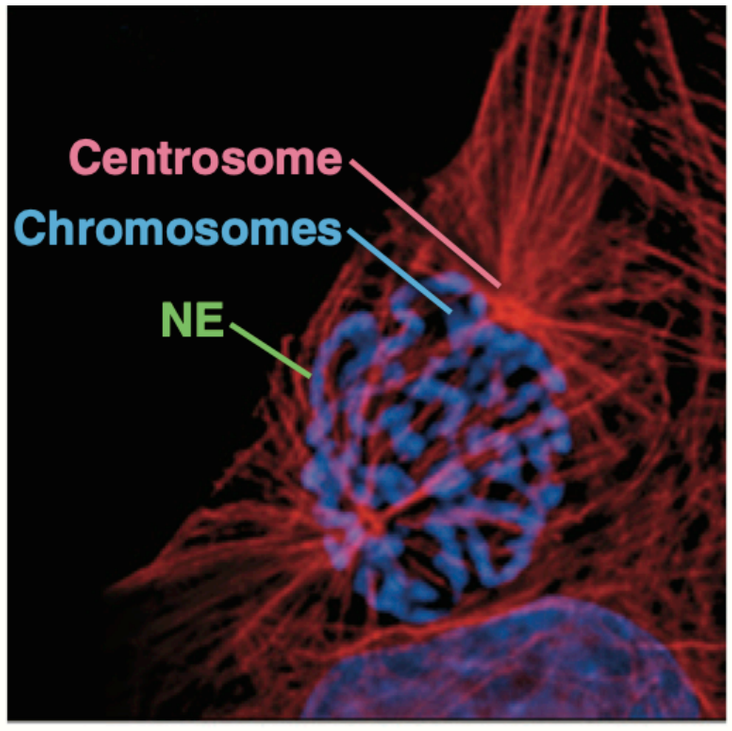
## Interphase




chromosomes duplicate

cell grows in size

## Prophase




chromosomes condense

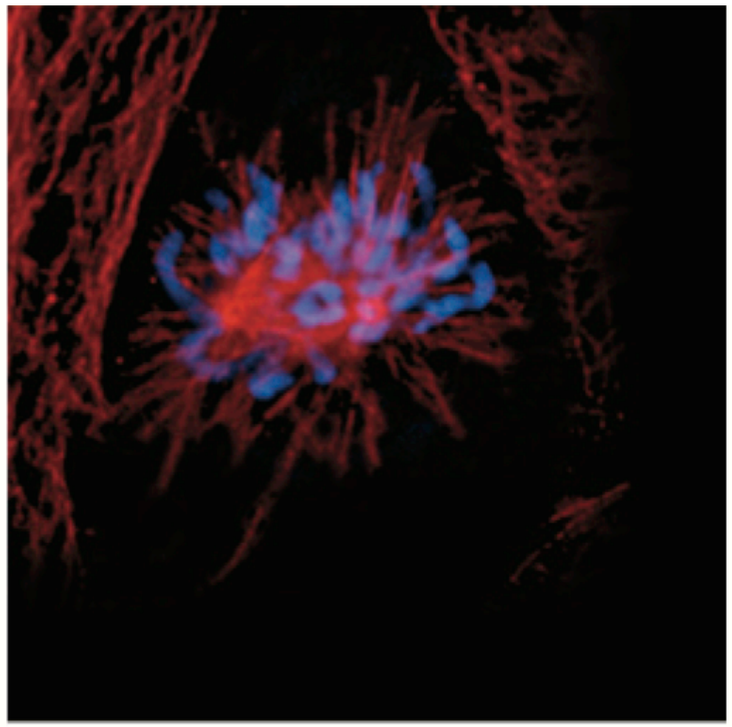
asters form around centrosomes

cell rounds up

Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

# *Morphological Changes During Mitosis*

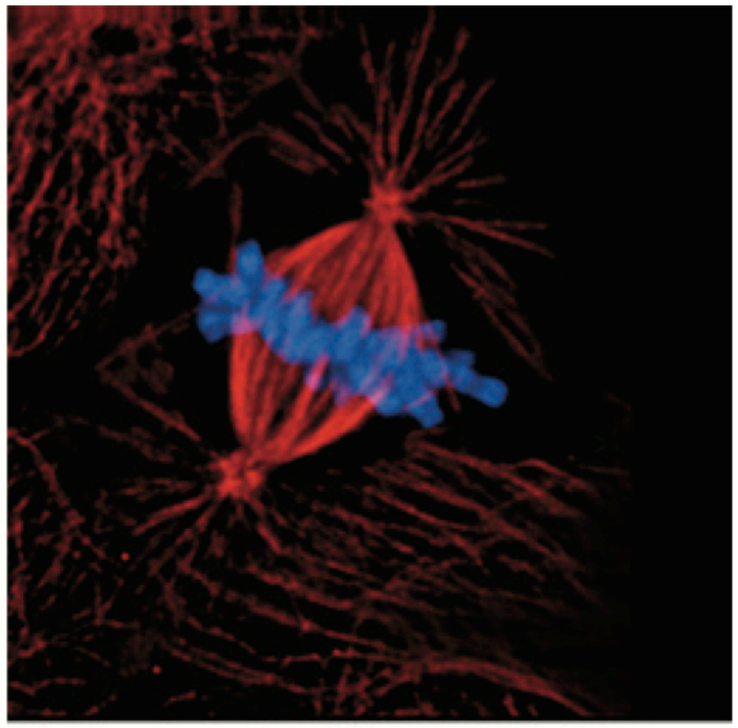
## Prometaphase



nuclear envelope breaks down

kinetochores capture microtubules

## Metaphase

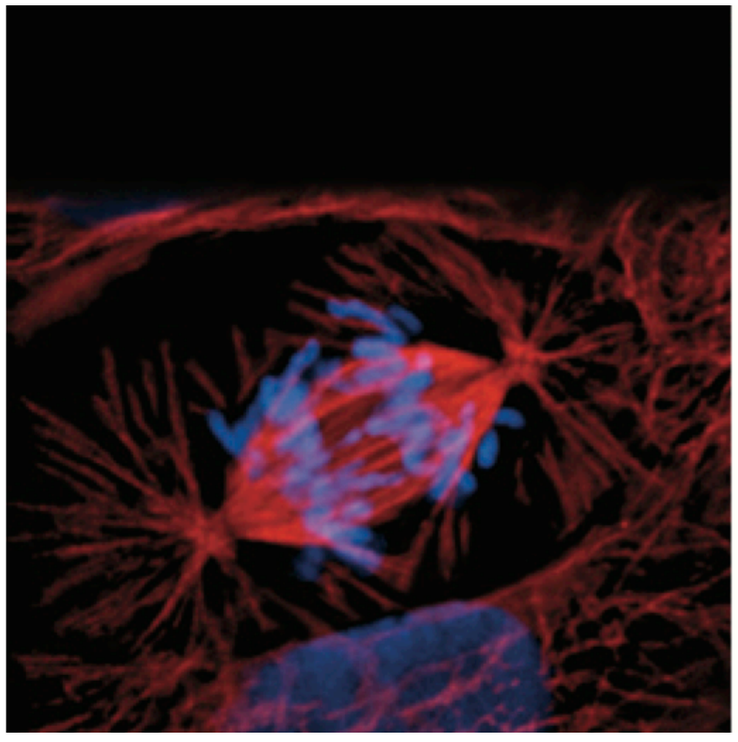


chromosomes align at spindle equator

Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

# *Morphological Changes During Mitosis*

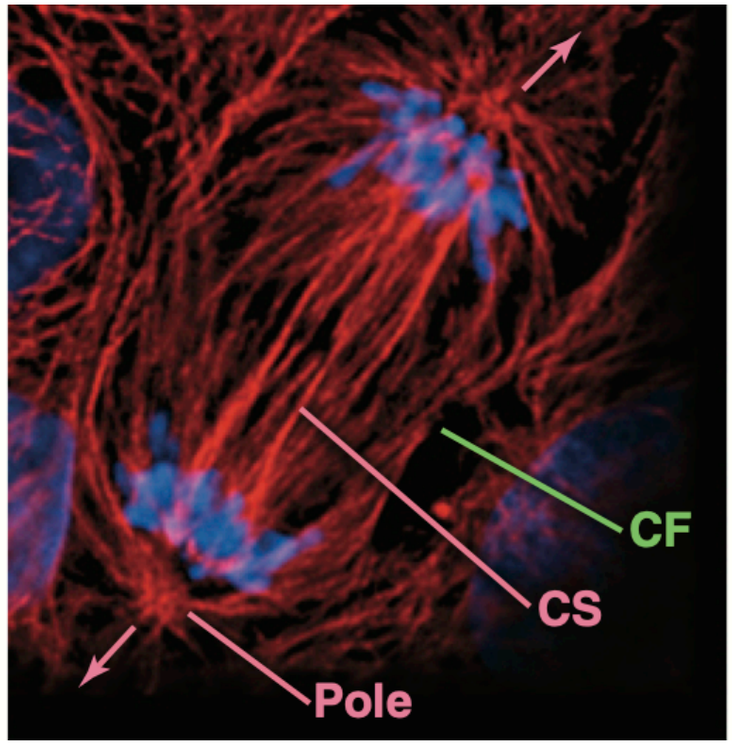
## Anaphase A



securin is degraded

sister chromatids move toward poles

## Anaphase B




central spindle (CS) assembles

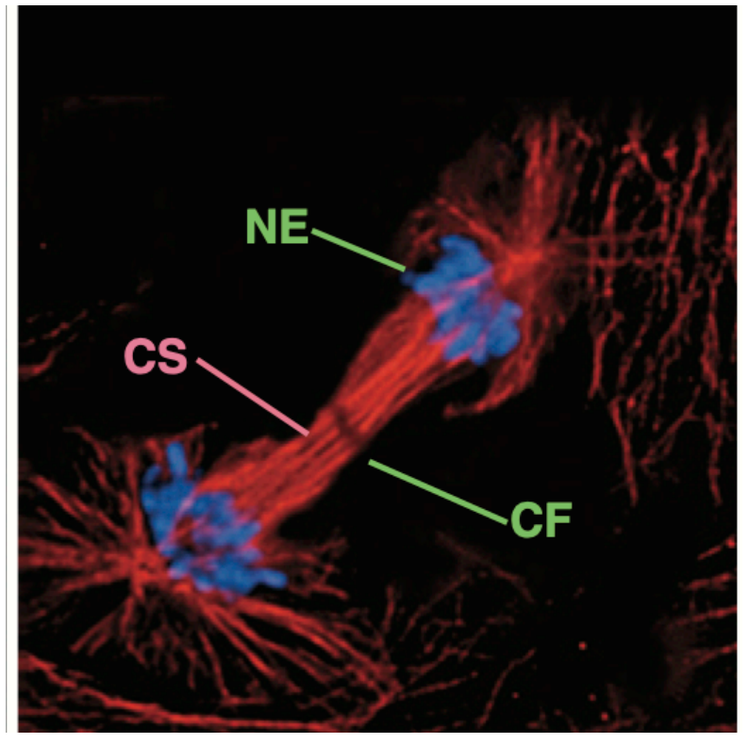
poles separate

cleavage furrow (CF) assembles

Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

# *Morphological Changes During Mitosis*

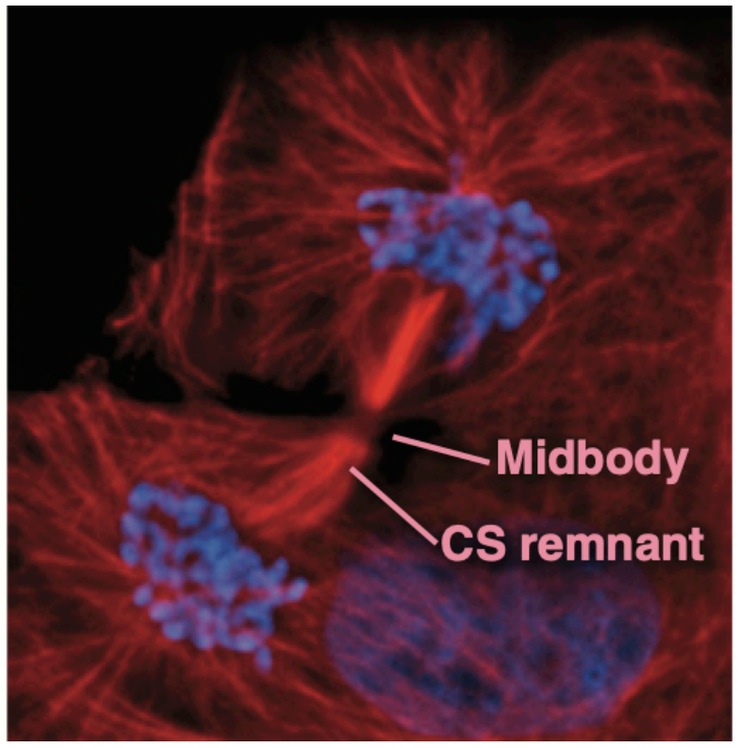
## Telophase




cleavage furrow (CF) constricts

nuclear envelope (NE) reassembles

## Cytokinesis



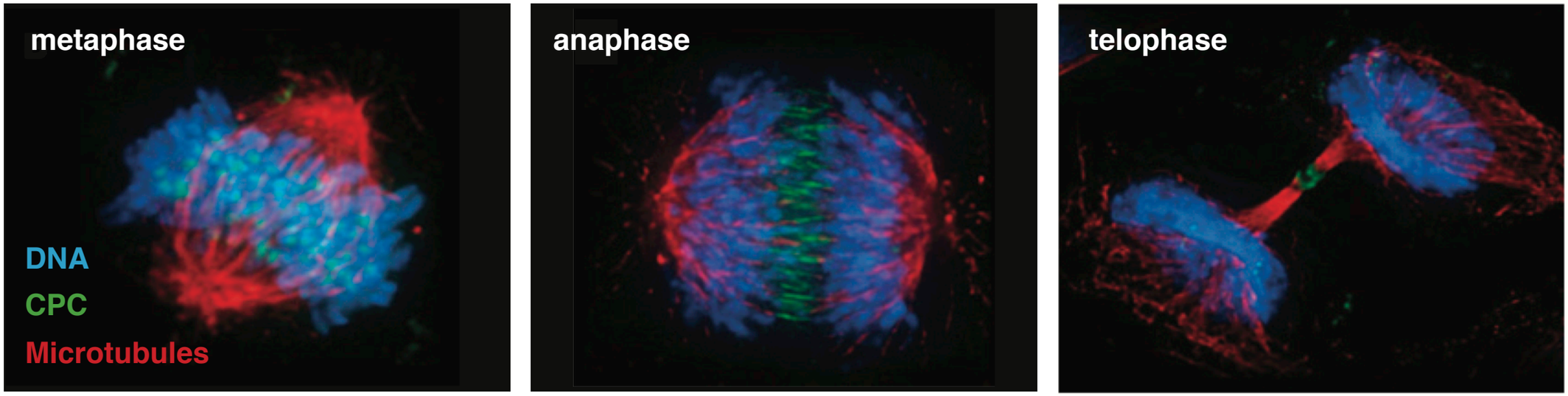

chromosomes decondense

microtubule cytoskeleton reassembles

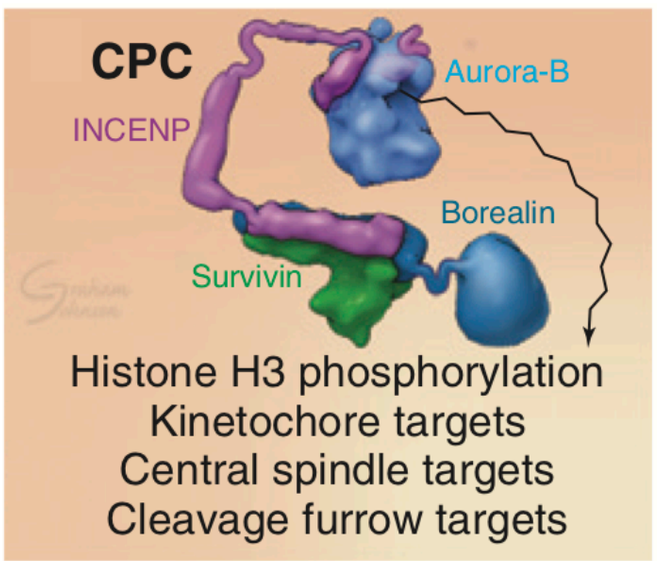
daughter cells separate

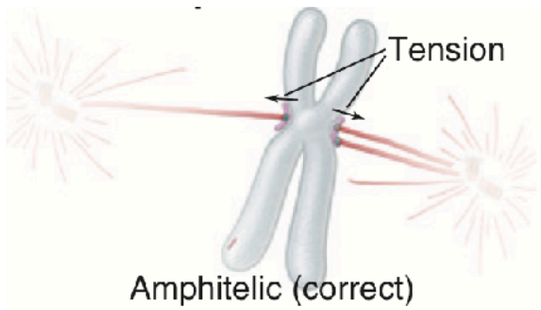
Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

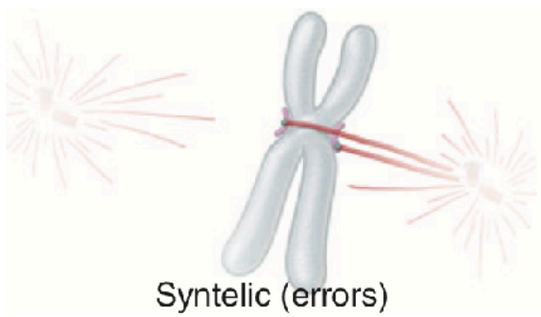
# *Chromosomal Passenger Complex (CPC) Corrects Kinetochore Attachment Errors*



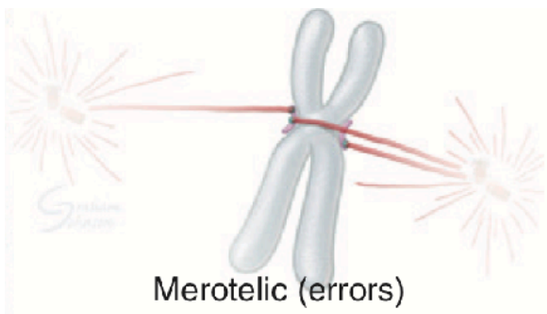

***What happens if spindle microtubules capture kinetochores incorrectly?***



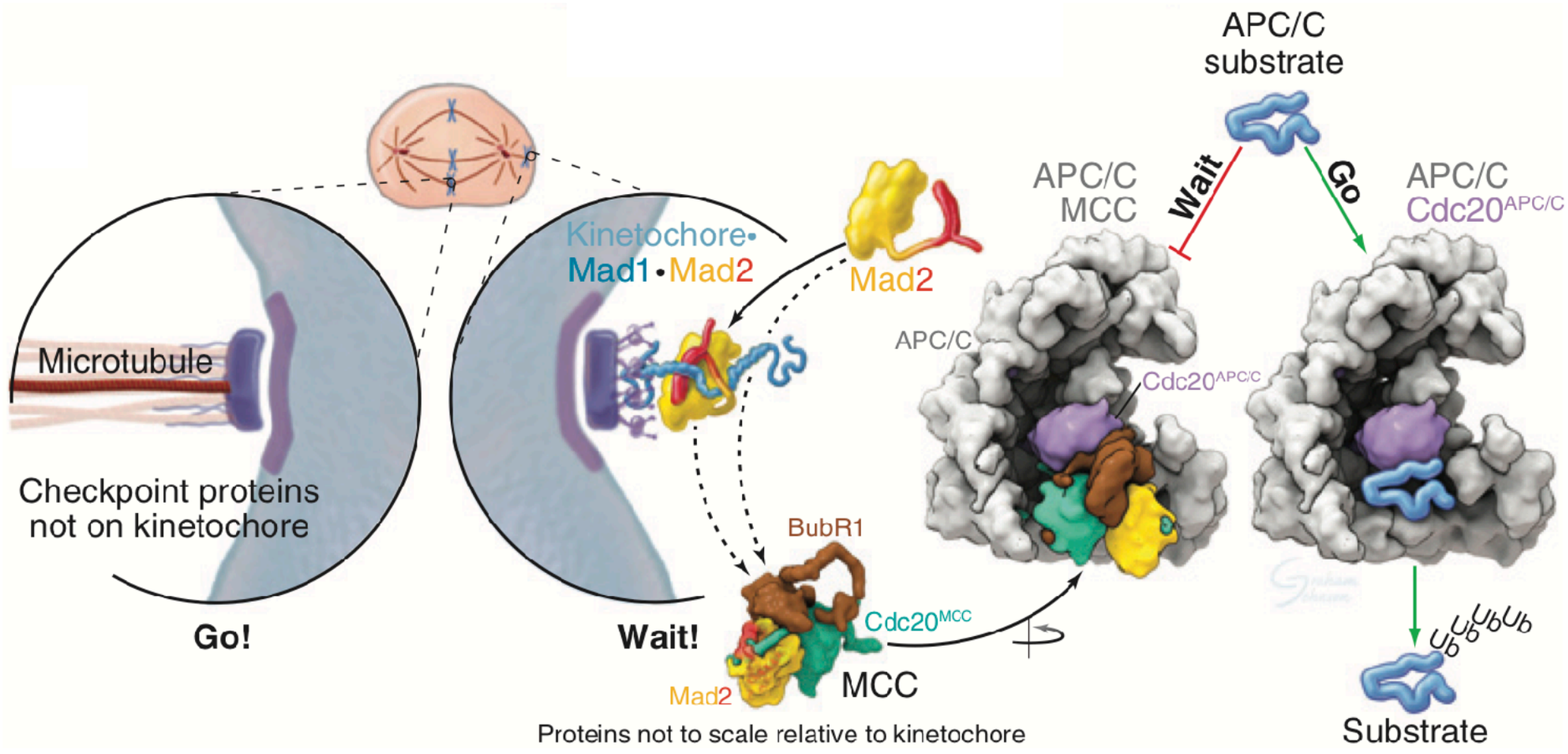




**Aurora B** phosphorylates kinetochore components, promoting dissociation



# *Spindle Assembly Checkpoint (SAC) Monitors Kinetochore Attachment*

*control of metaphase/anaphase transition by the SAC*




1. **Aurora B kinase** signaling (not shown) recruits **Mad1/2** to the unattached kinetochore
2. The **mitotic checkpoint complex** (MCC) assembles, inhibiting the **APC/C** (E3 ubiqitin ligase)
3. Kinetochore attachment frees APC/C to target substrates for onset of anaphase (cyclin B, securin)

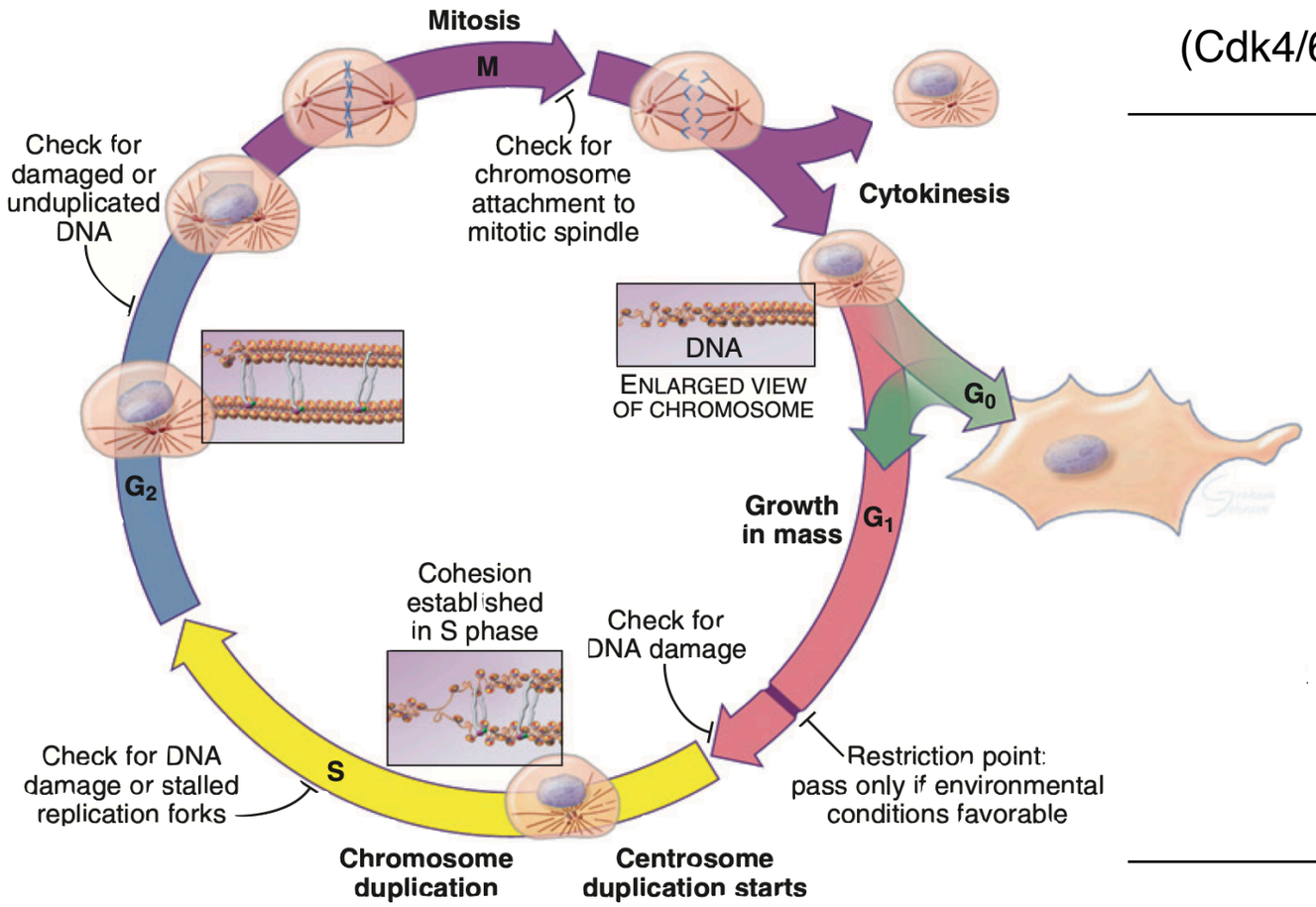
Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

# *Targeting Cell Cycle Proteins for Cancer Therapeutics*

**Induce genome instability**

e.g., barasertib

(Aurora B inhibitor)




**Force cell cycle exit**

e.g., palbociclib

(Cdk4/6 inhibitor)

**Exploit replication stress**

e.g., prexasertib

(Chk1 inhibitor)

**Force cycle progression**

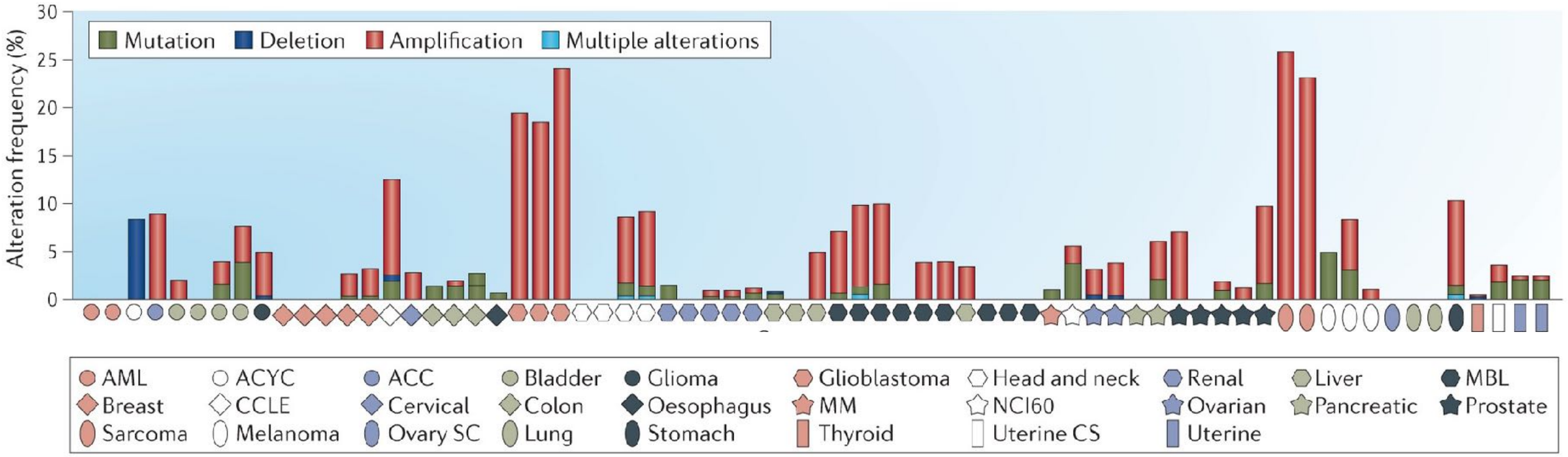
e.g., adavosertib

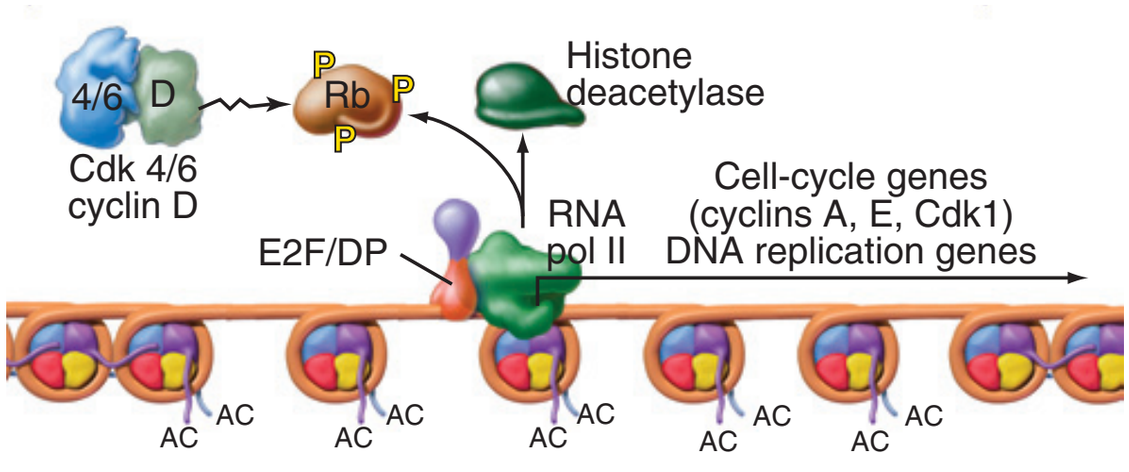
(Wee1 inhibitor)

Pollard, T. D.; Earnshaw, W. C.; Lippincott-Schwartz, J. Johnson, G. T. *Cell Biology,* 3rd ed.; Elsevier: Philadelphia, **2017**.

# *Cdk4/6 Inhibitors Force Cell Cycle Exit*

- Cdk4/6 very commonly upregulated in cancers



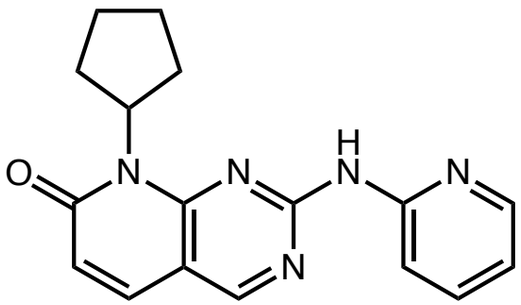




*selective inhibition of Cdk4/6 activity should induce cytostatic $G_0/G_1$ arrest*

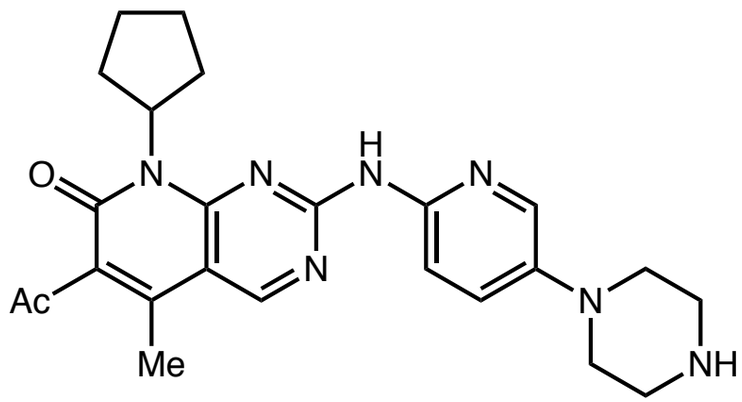
Toogood, P. L. et al. *J. Med. Chem.* **2005**, *48,* 2388.

# *Cdk4/6 Inhibitors Force Cell Cycle Exit*

- Pyrido[2,3-*d*]pyrimidin-7-one scaffold provided highly selective inhibition for Cdk4/6 over other Cdks



**hit compound**



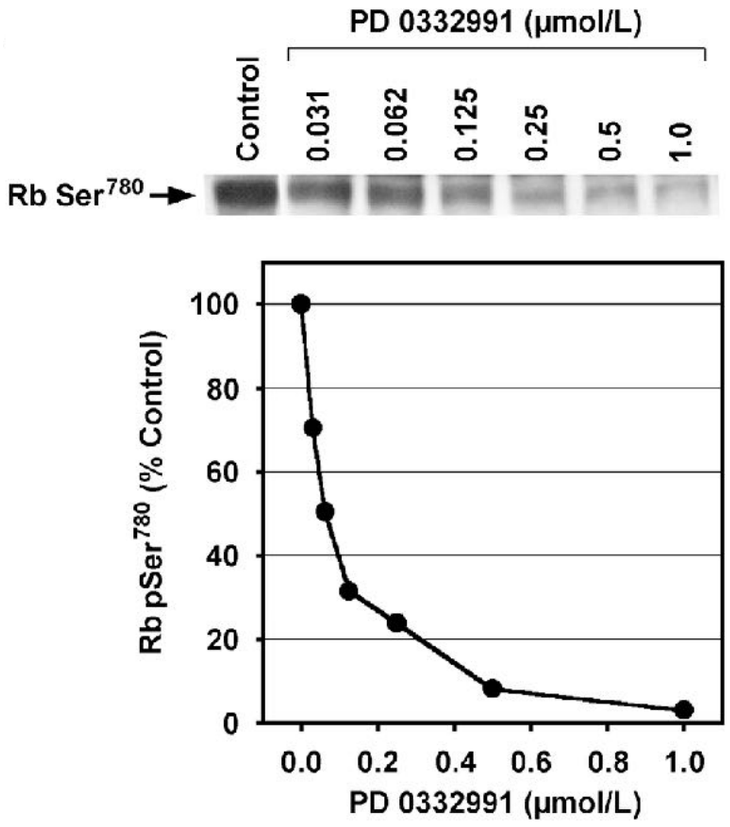
**PD-0332991 (palbociclib)**

| *assay via* *[$\gamma$-$^{32}$P]ATP* *incorporation* | | |
|---|---|---|
| **Cdk4–cyclin D** $IC_{50}$ ($\mu$M) | 0.145 | 0.011 |
| **Cdk2–cyclin A** $IC_{50}$ ($\mu$M) | 5.010 | 5.010 |

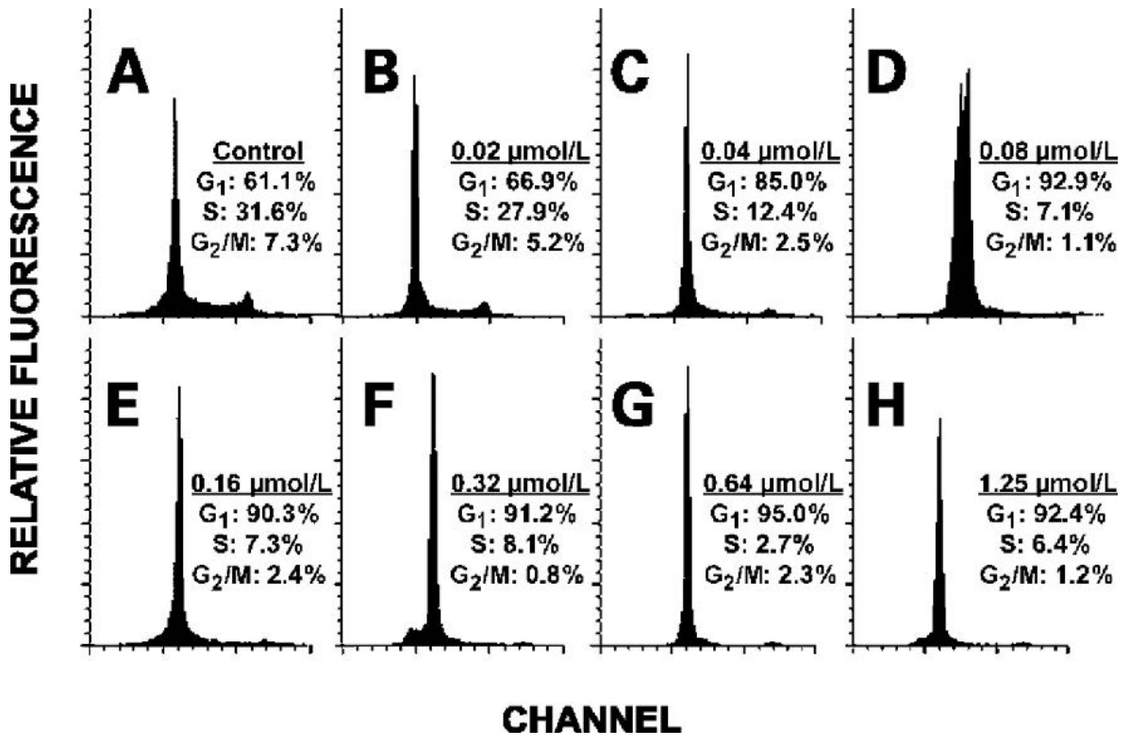
Toogood, P. L. et al. *J. Med. Chem.* **2005**, *48,* 2388.

# *Cdk4/6 Inhibitors Force Cell Cycle Exit*

- In vivo studies with human tumor xenografts showed promising tumor regression and confirmed MoA




inhibition of Rb phosphorylation
at Cdk4-specific sites



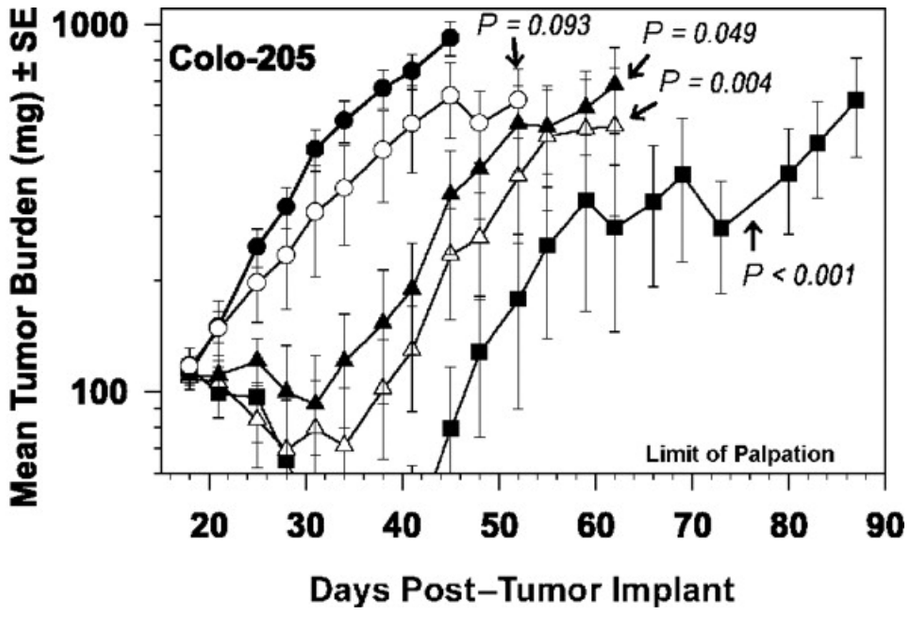

antiproliferative activity arrests
human breast carcinoma cells in $G_1$

Fry, D. W. et al. *Mol. Cancer Ther.* **2004**, *3,* 1427.

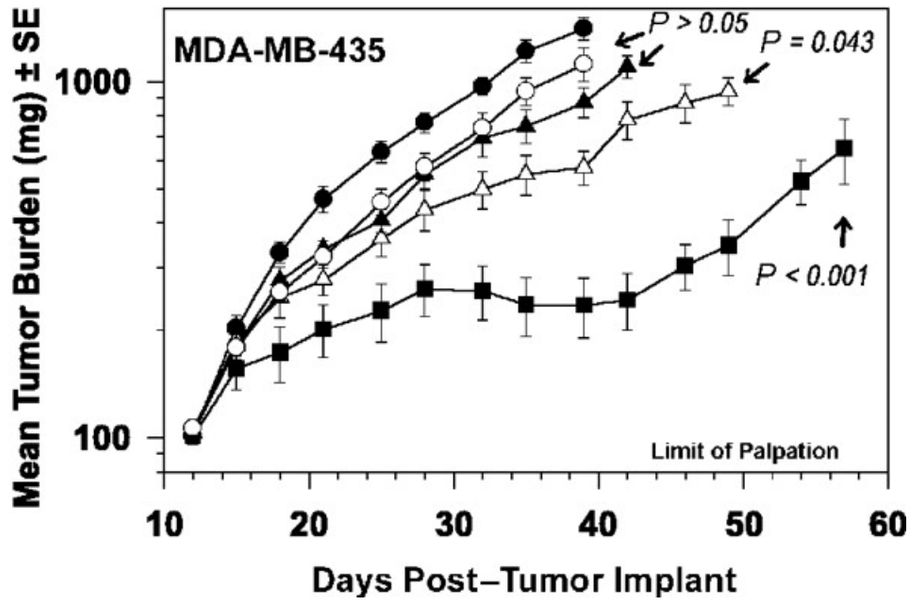
# *Cdk4/6 Inhibitors Force Cell Cycle Exit*

- In vivo studies with human tumor xenografts showed promising tumor regression and confirmed MoA

human colon carcinoma




human breast carcinoma

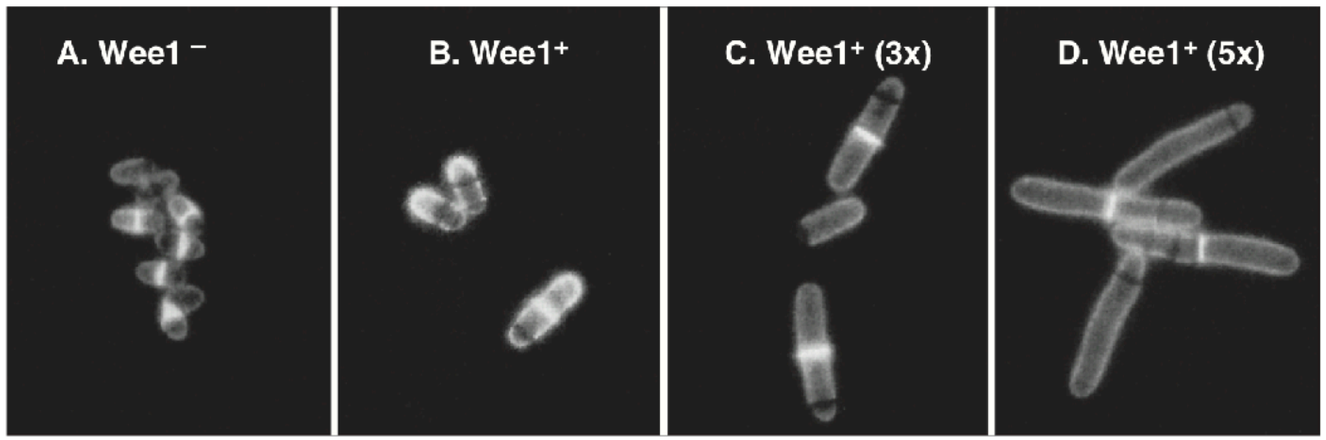



**approved in February 2015** for ER$^{+}$ Her2$^{-}$ advanced breast cancer in combination with letrozole (aromatase inhibitor)

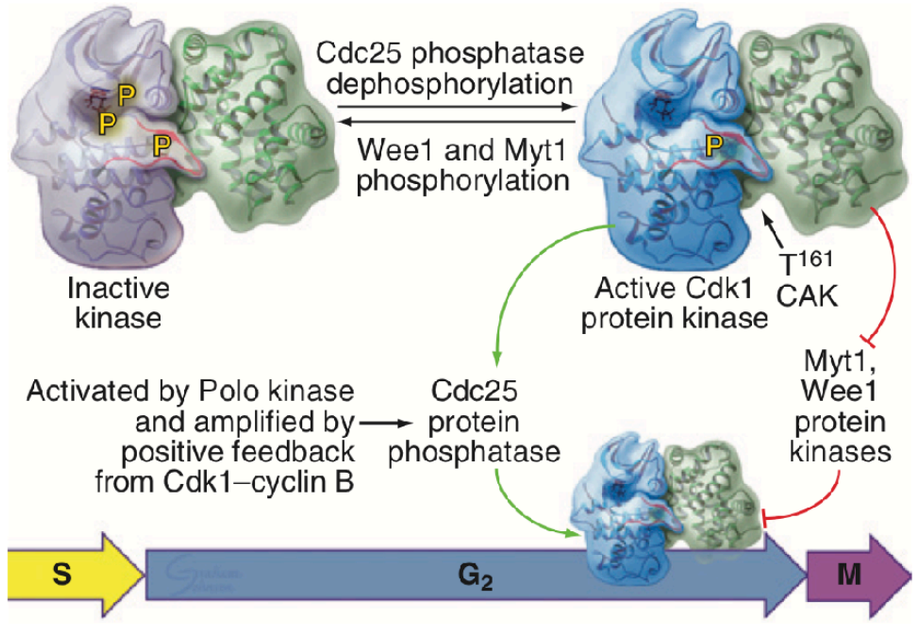
Fry, D. W. et al. *Mol. Cancer Ther.* **2004**, *3,* 1427.

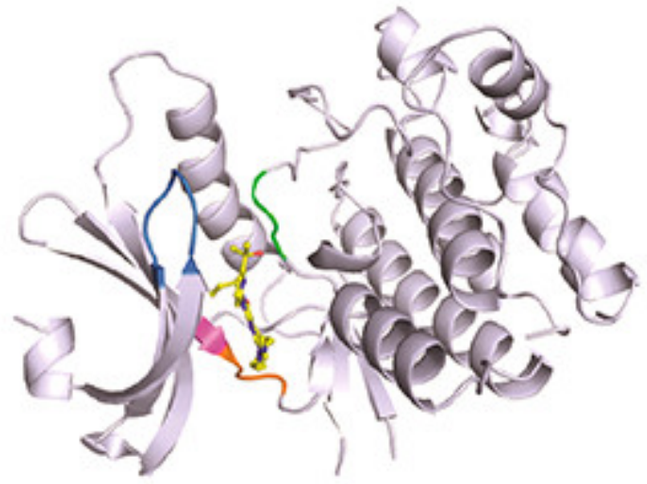
# *Wee1 Inhibitors Force Cell Cycle Progression*

- Strategy targets regulation of the $G_2$/M transition, forcing premature cell cycle progression




Wee1 restricts $G_2$/M transition by phosphorylating Cdk1 (Cdc2) at Y15

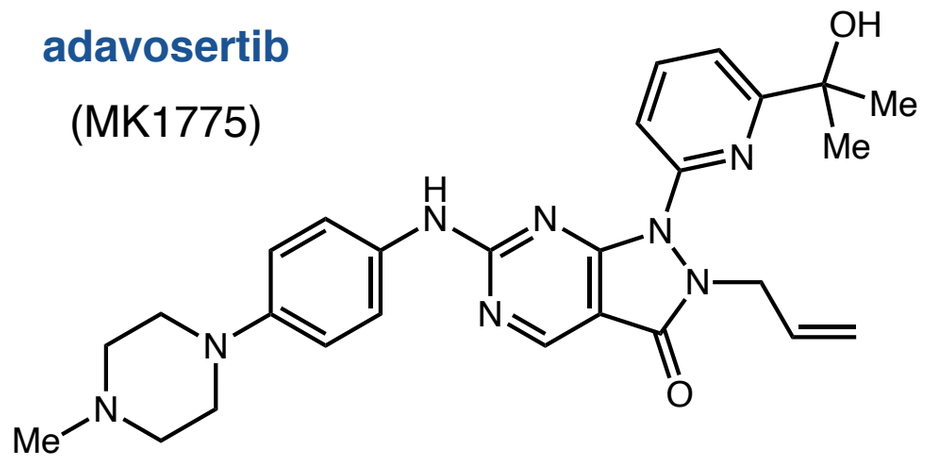


Wee1•adavosertib

**adavosertib**

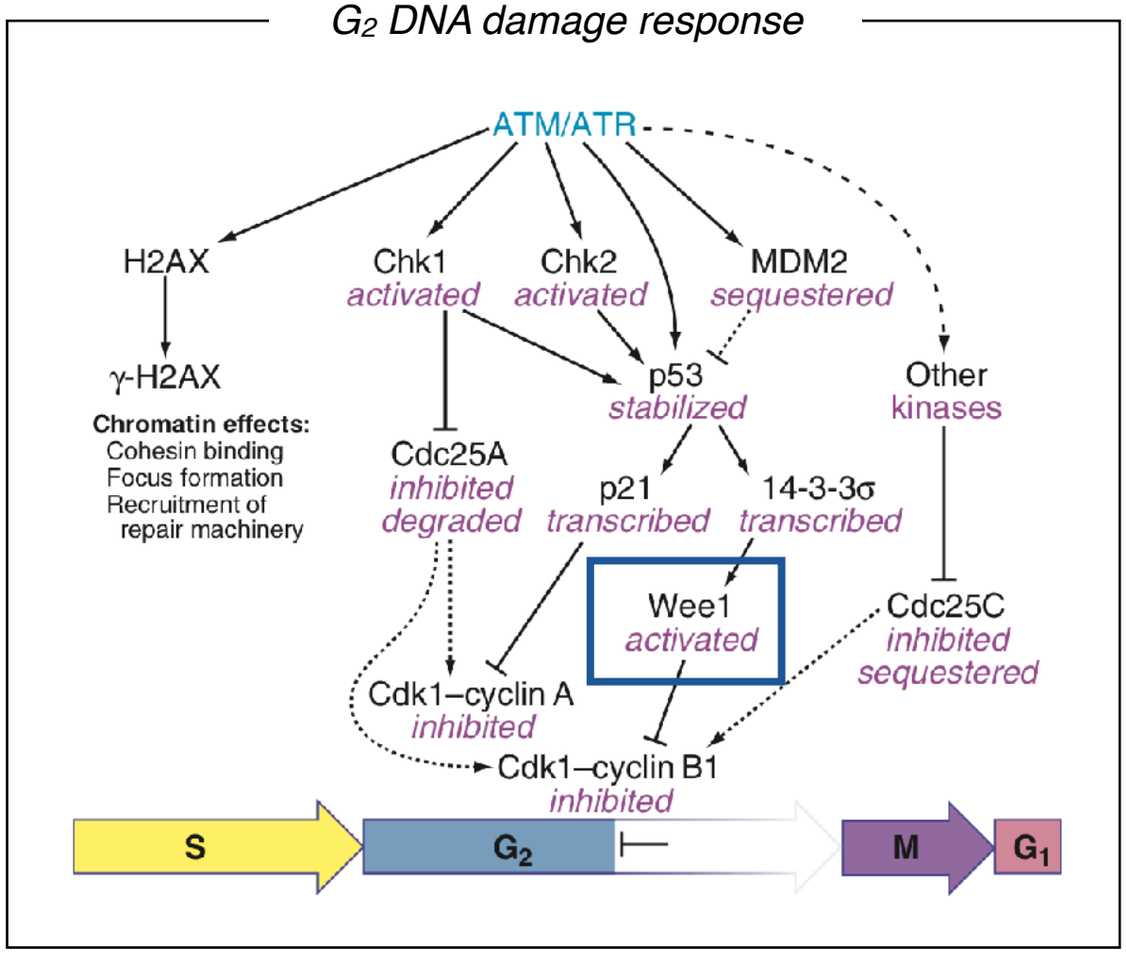
(MK1775)

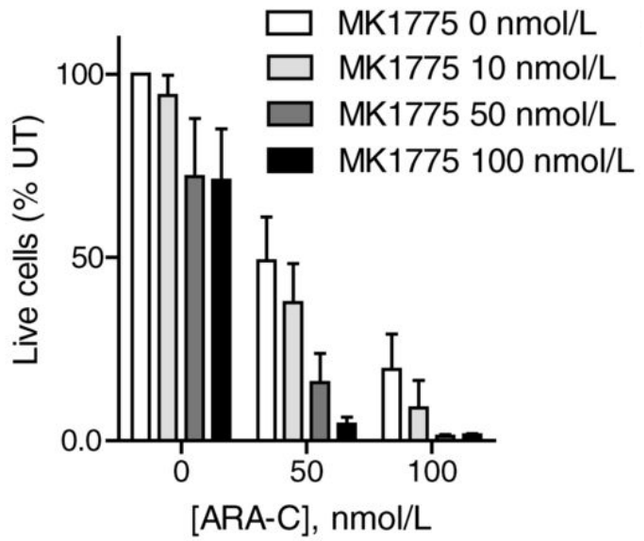


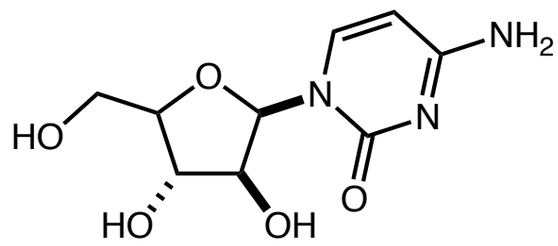

Zhu, J.-Y. et al. *J. Med. Chem.* **2017**, *60,* 7863.

# *Wee1 Inhibitors Force Cell Cycle Progression*

- Wee1 inhibition permits sensitization to co-dosed genotoxics by suppressing DNA damage response



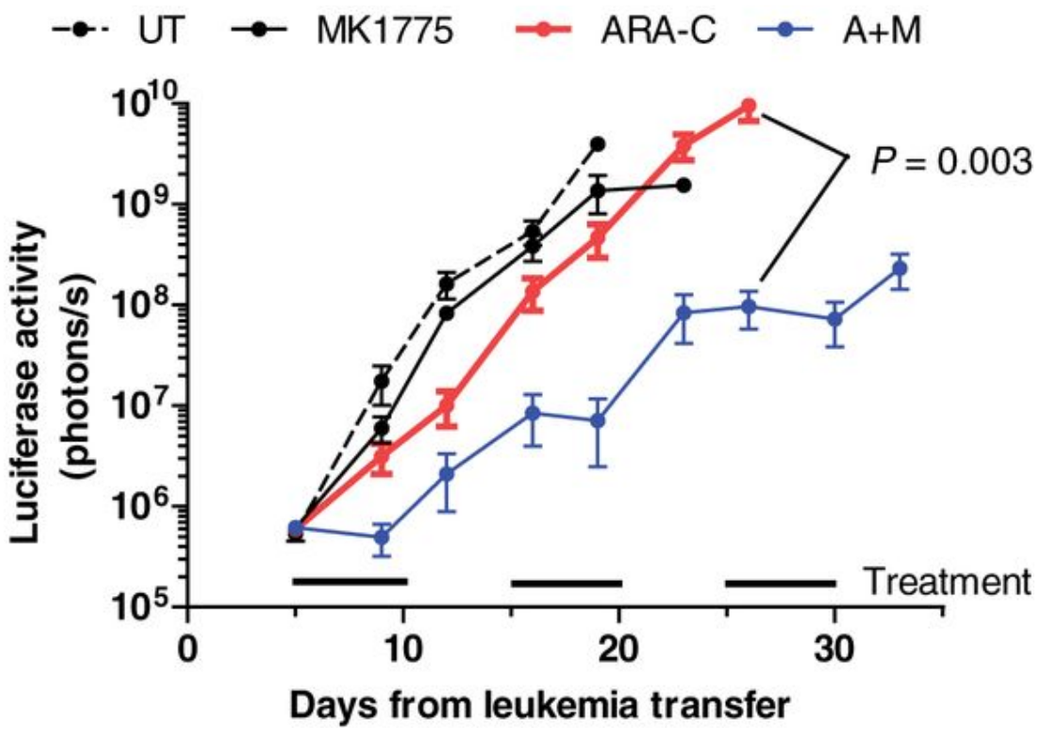

cytosine arabinoside

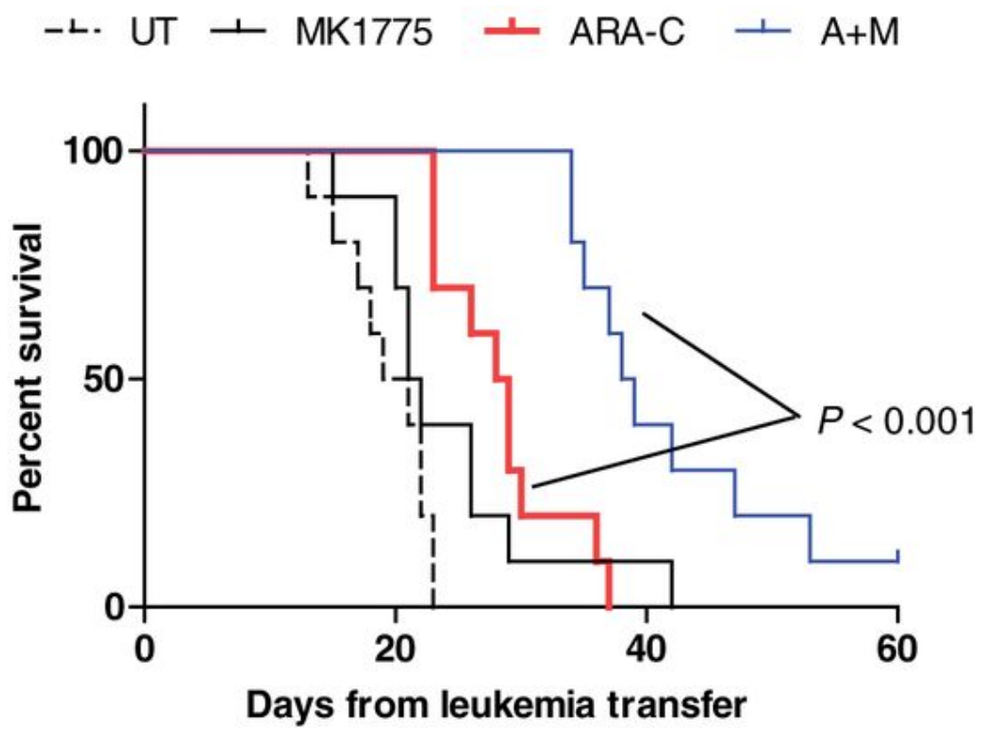
(ara-C, cytarabine)

Linden, A. A. V.; Baturin, D.; Ford, J. B.; Fosmire, S. P.; Gardner, L.; Korch, C.; Reigan, P.; Porter, C. C. *Mol. Cancer Ther.* **2013**, *12,* 2675.

# *Wee1 Inhibitors Force Cell Cycle Progression*

- Wee1 inhibition permits sensitization to co-dosed genotoxics by suppressing DNA damage response



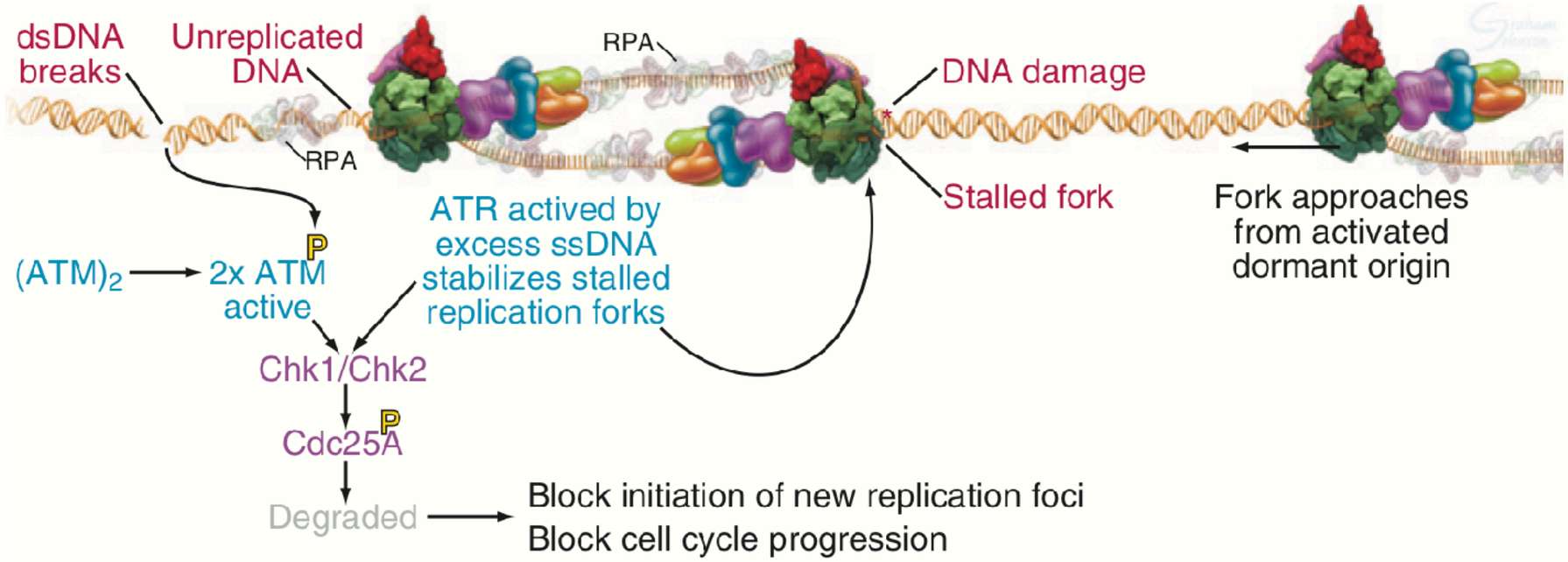




MK-1775 was licensed to AstraZeneca (AZD-1775, **adavosertib**) in 2013, with ~60 phase I/II clinical trials currently ongoing for various cancer types

Hirai, H. et al. *Cancer Bio. Ther.* **2010**, *9*, 514.

# *Chk1 Inhibitors Impair Oncogenic Replication Stress Tolerance*

- **ATR** and **Chk1** are two promising targets associated with oncogene-induced DNA replication stress

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Actively dividing cancer cells experience higher levels of **replication stress** (ssDNA) and are more susceptible to **replication catastrophe**

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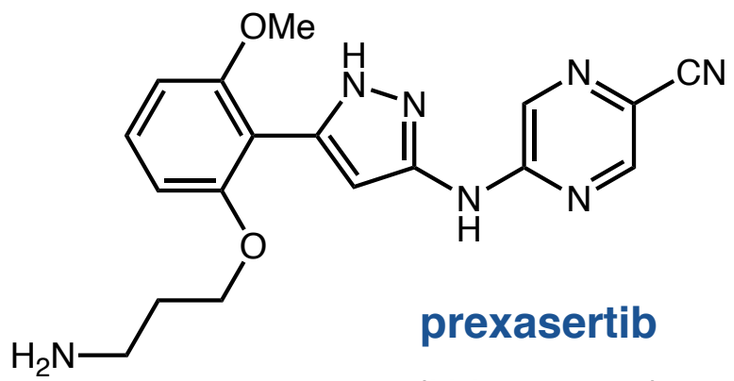


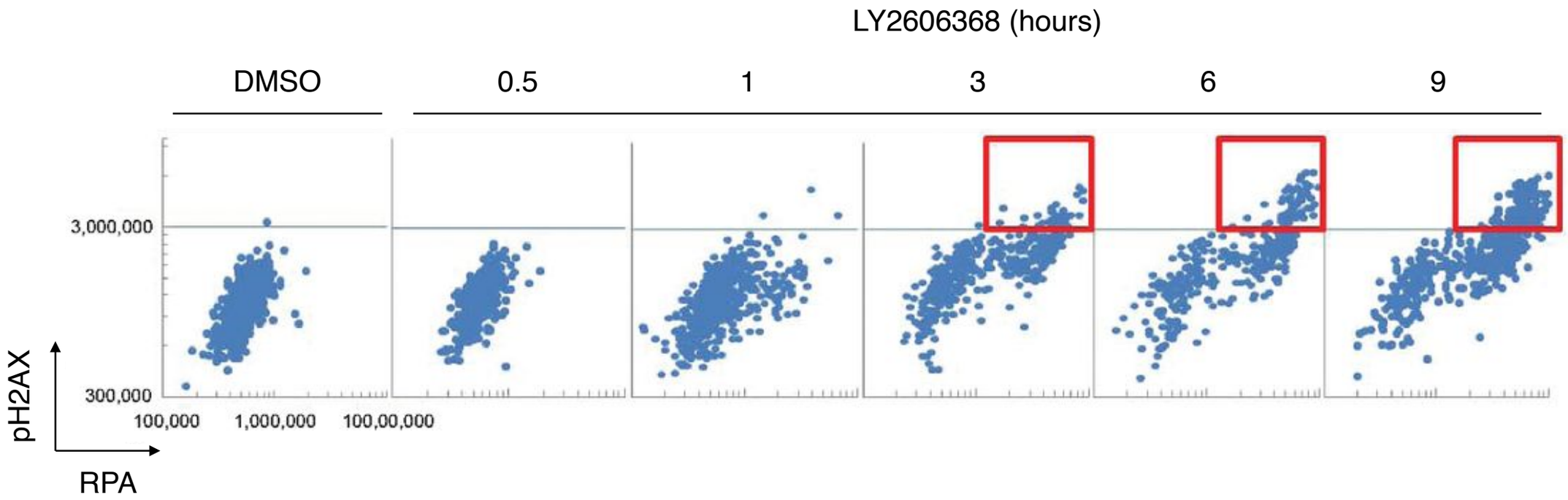

**prexasertib**

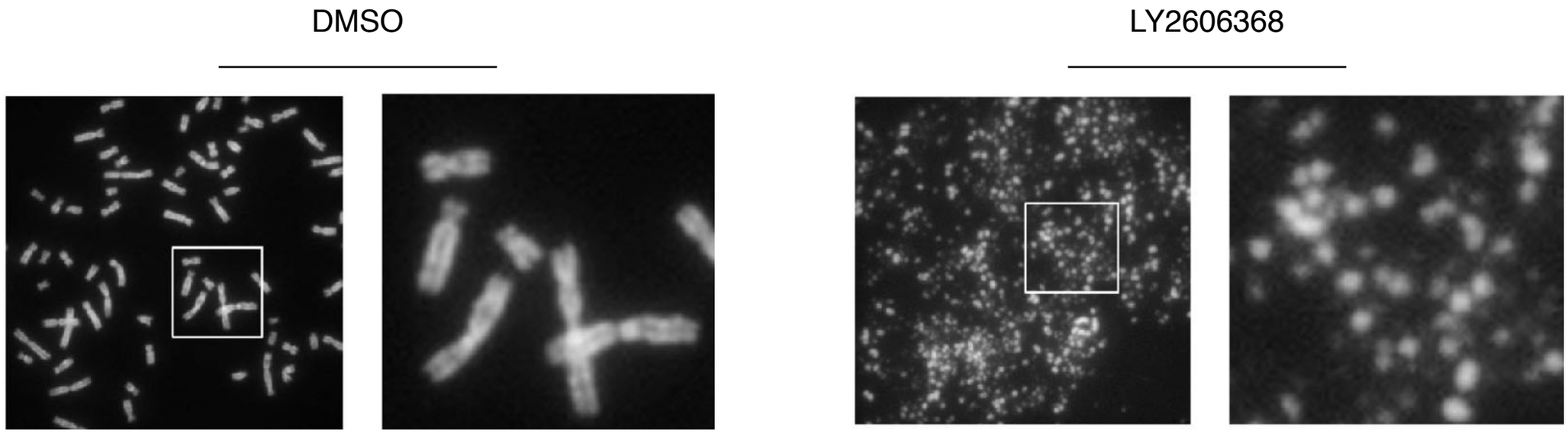
(LY2606368)

King, C.; Diaz, H. B.; McNeely, S.; Barnard, D.; Dempsey, J.; Blosser, W.; Beckmann, R.; Barda, D.; Marshall, M. S. *Mol. Cancer Ther.* **2015**, *14,* 2004.

# *Chk1 Inhibitors Impair Oncogenic Replication Stress Tolerance*

- **Chk1** inhibition by LY2606368 causes accumulation of DNA damage and replication catastrophe

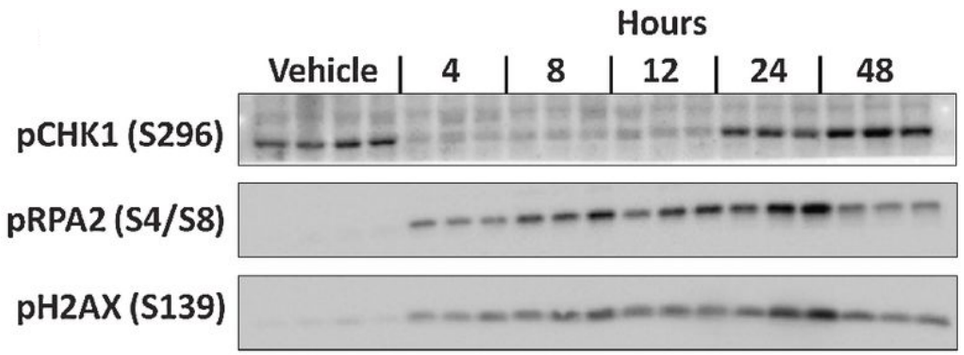


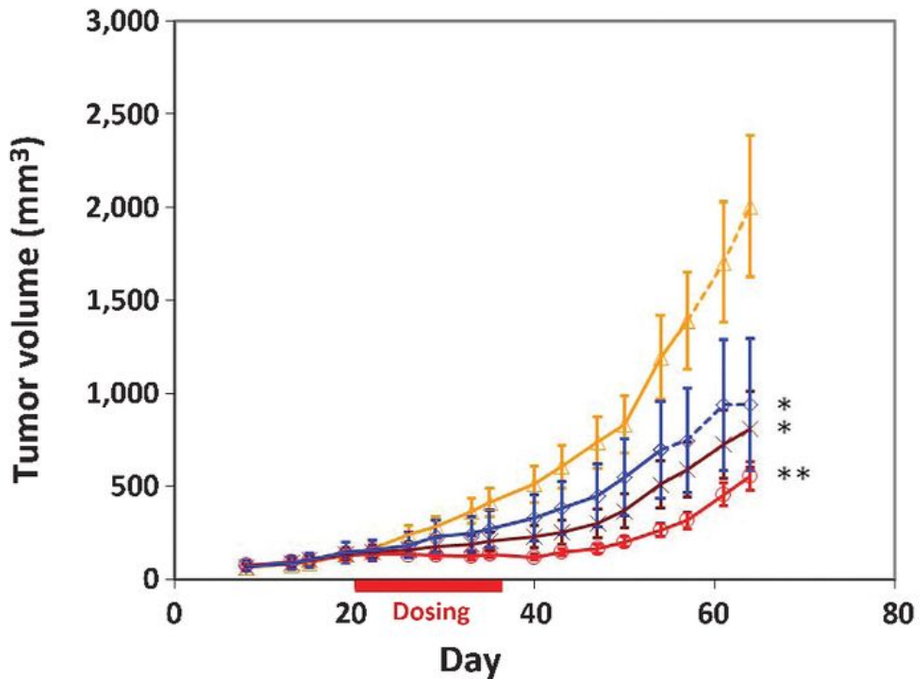




King, C.; Diaz, H. B.; McNeely, S.; Barnard, D.; Dempsey, J.; Blosser, W.; Beckmann, R.; Barda, D.; Marshall, M. S. *Mol. Cancer Ther.* **2015**, *14,* 2004.

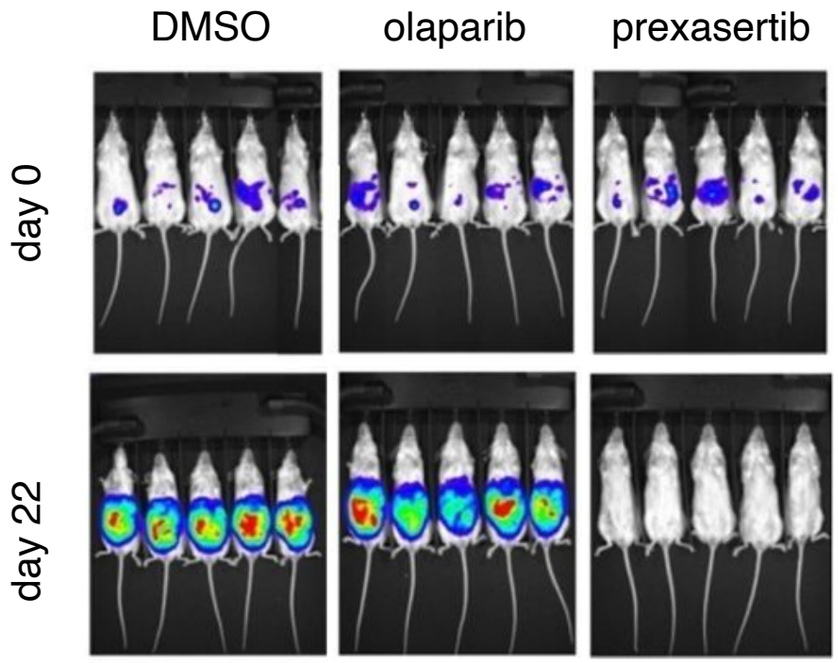
# *Chk1 Inhibitors Impair Oncogenic Replication Stress Tolerance*

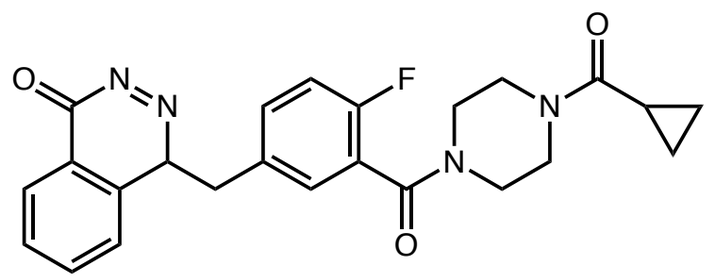
- Prexasertib induces DNA damage in tumors and inhibits lung carcinoma in xenograft model




- Activity in PARP-inhibitor resistant ovarian cancer



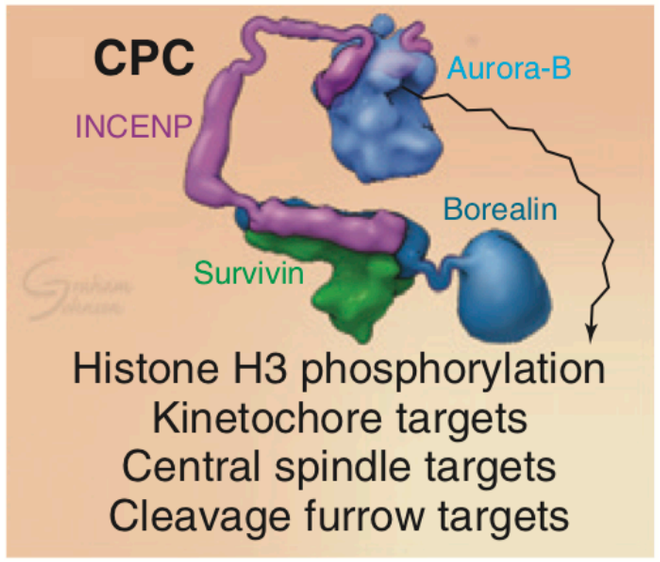

**olaparib (Lynparza) – PARP inhibitor**

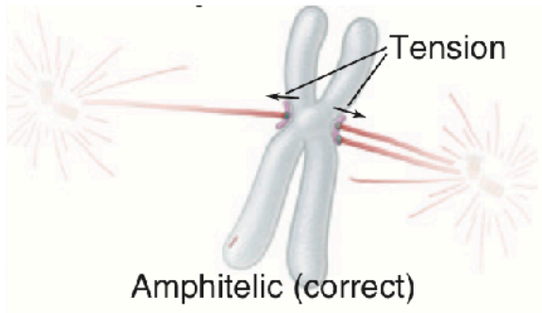
poly(ADP-ribose) polymerase critical for genome stability

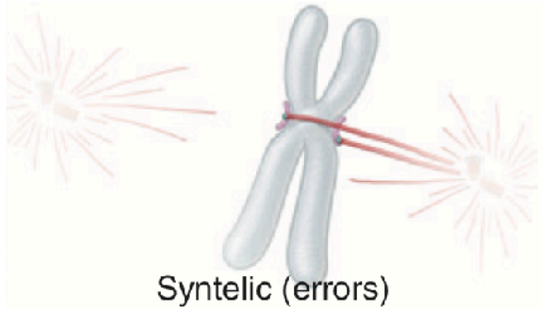
King, C.; Diaz, H. B.; McNeely, S.; Barnard, D.; Dempsey, J.; Blosser, W.; Beckmann, R.; Barda, D.; Marshall, M. S. *Mol. Cancer Ther.* **2015**, *14,* 2004.
Parmar, K. et al. *Clin. Cancer Res.* **2019**, *25,* 6127.

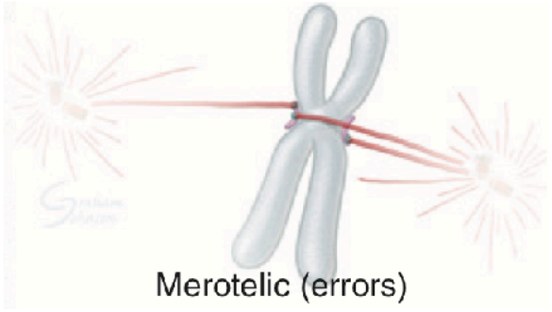
# *Aurora B Inhibitors Interfere with Chromosomal Segregation*

- Inhibitors of the mitotic spindle and spindle assembly checkpoint induce chromosome mis-segregation

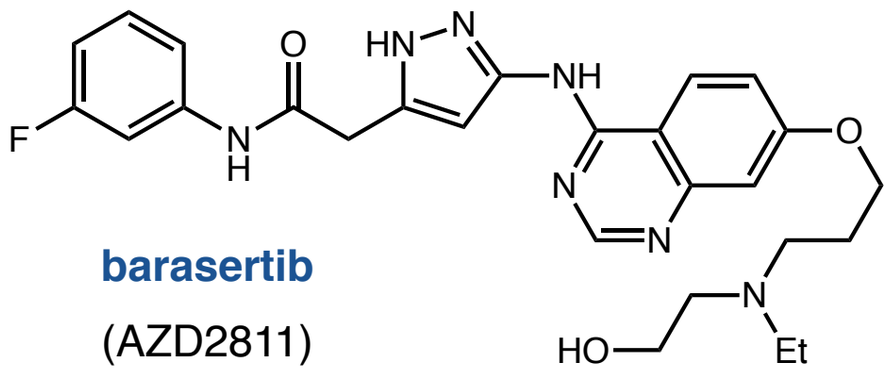



**Aurora B** phosphorylates kinetochore components, promoting dissociation




**barasertib**

(AZD2811)

in vitro selectivity

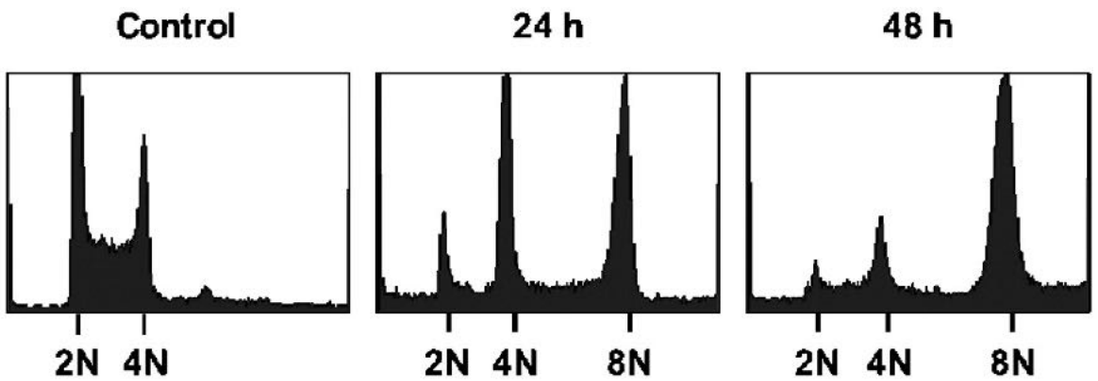
| kinase | $IC_{50}$, $\mu M^a$ | kinase | $IC_{50}$, $\mu M^a$ |
|---|---|---|---|
| Aurora A | 1.4 | KDR | 1.8 |
| Aurora B-INCENP | <0.001 | PHK | 1.8 |
| Aurora C-INCENP | 0.017 | ZAP70 | 8.2 |
| LCK | 0.17 | others[b] | >10 |

Mortlock, A. A. et al. *J. Med. Chem.* **2007**, *50,* 2213.

# *Aurora B Inhibitors Interfere with Chromosomal Segregation*

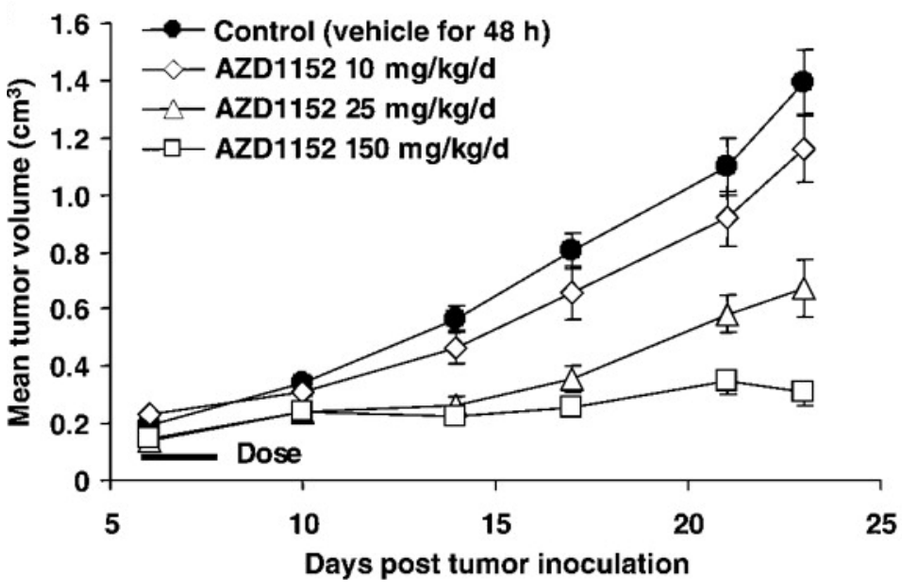
- Aurora B inhibitor barasertib induces polyploidy and inhibits cancer growth in xenograft models

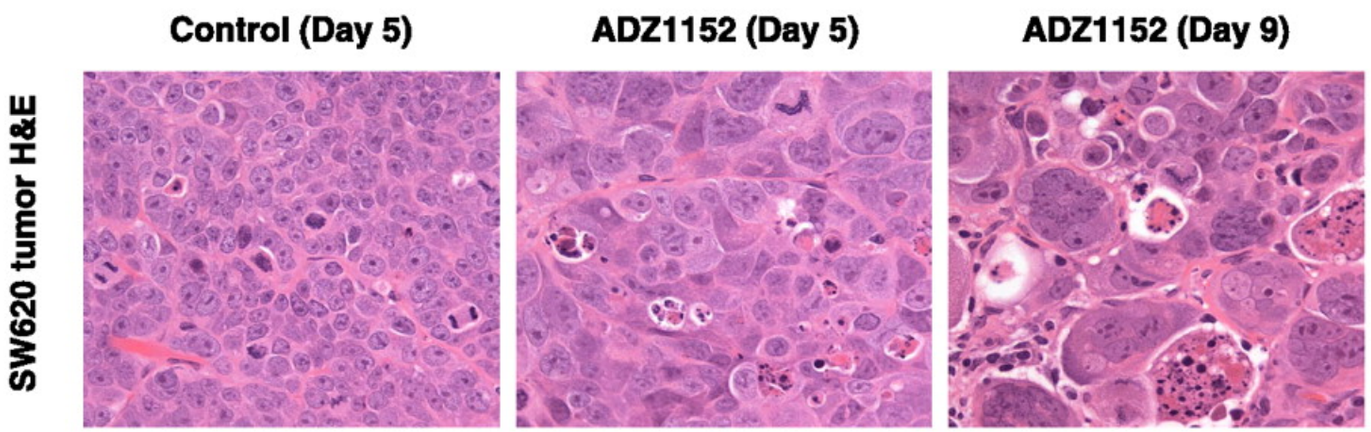
**DNA content in treated cells**




DNA replicates but cells do not divide

**inhibition of colorectal cancer xenografts**

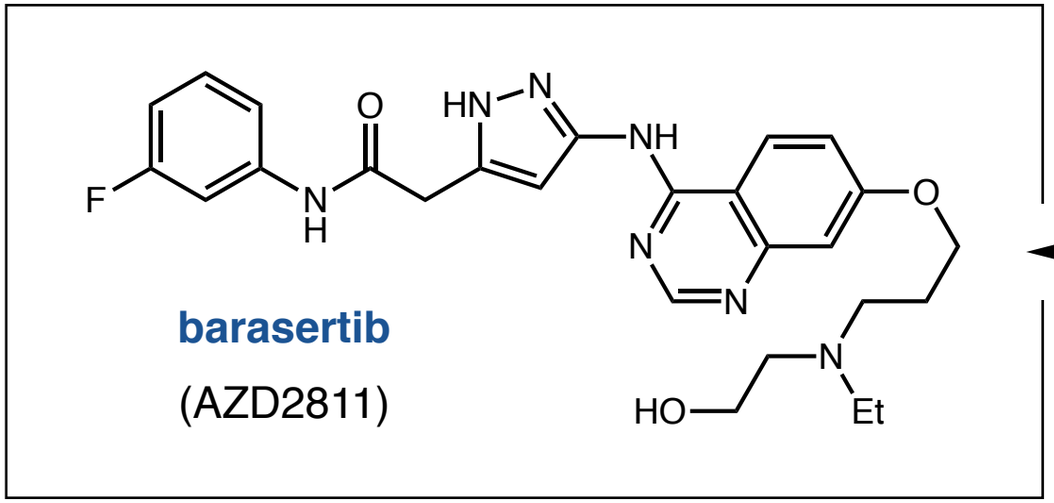



histology consistent with apoptotic induction in cancer tissues

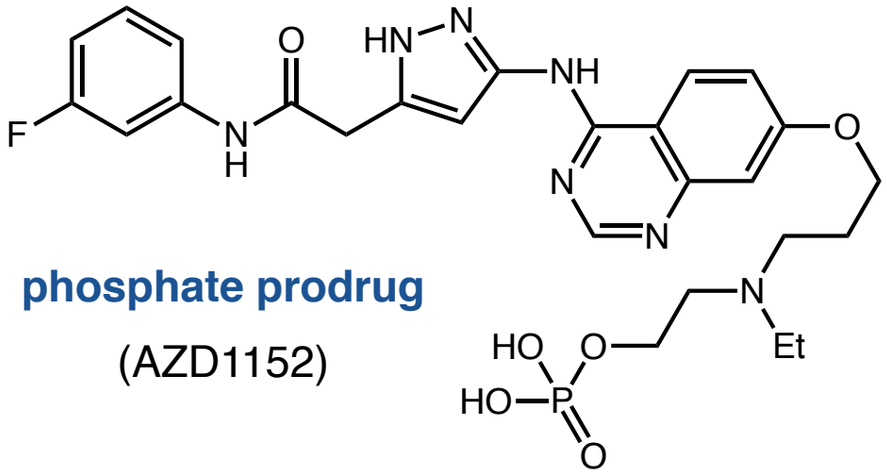

Helfrich, B. A.; Kim, J.; Gao, D.; Chan, D. C.; Zhang, Z.; Tan, A.-C.; Bunn, P. A. *Mol. Cancer Ther.* **2015**, *15,* 2314.
Wilkinson, R. W. et al. *Clin. Cancer Res.* **2007**, *13,* 3682.

# *Aurora B Inhibitors Interfere with Chromosomal Segregation*



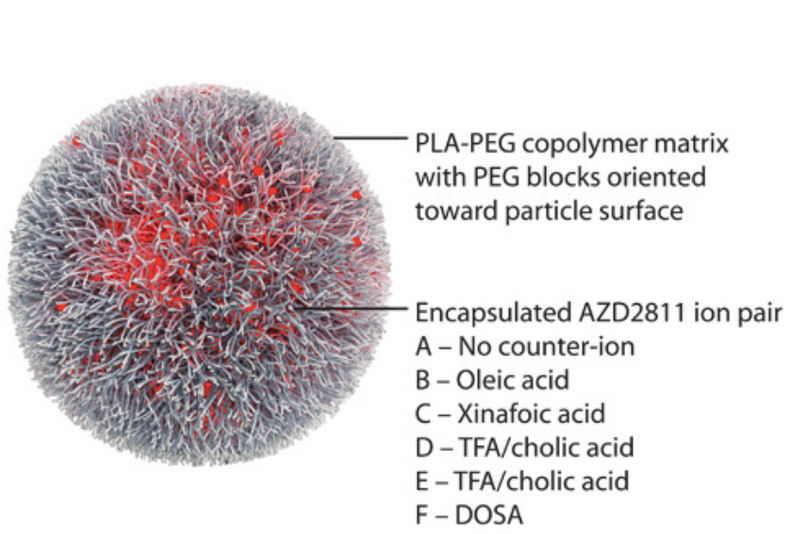

**barasertib**

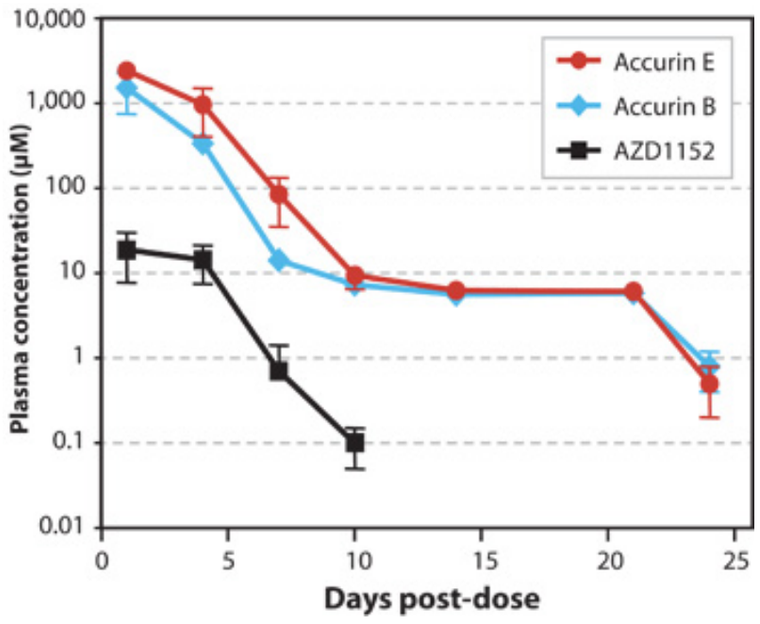
(AZD2811)




**phosphate prodrug**

(AZD1152)




nanoparticle formulation provides better safety profile than prodrug and good release kinetics

*Ongoing trials in phase I/II for hematological cancers, small-cell lung cancer, and prostate cancer*


Ashton, S. et al. *Sci. Transl. Med.* **2016**, *8,* 325ra17.
Floc'h, N. et al. *Mol. Cancer Ther.* **2019**, *18,* 909.