Aneuploidy and Genomic Instability

PATH 4500 Lecture

Fall 2025

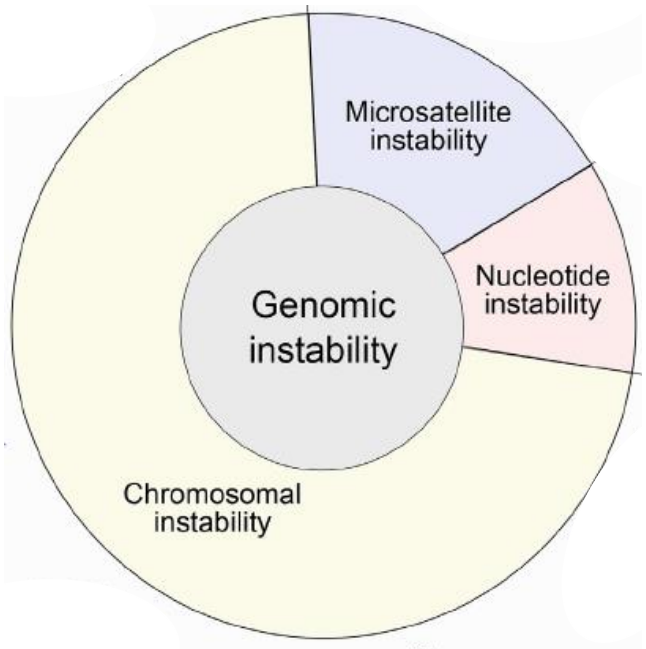
Alison M. Taylor, PhD

Outline for today

1. What is genomic instability and chromosome instability? What causes them to occur?
2. What are some tools the cell uses to prevent genomic and chromosome instability (CIN)?
3. What are the consequences of CIN in cancer?
4. How can we harness this therapeutically?

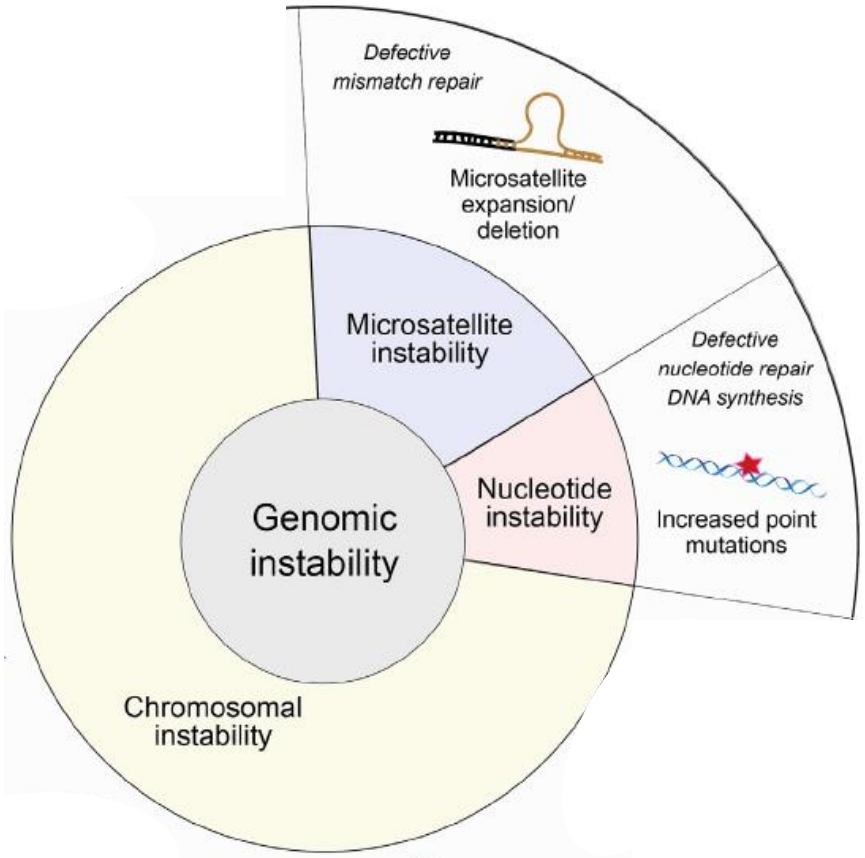
What types of genomic alterations occur in cancer?

What is genomic instability?



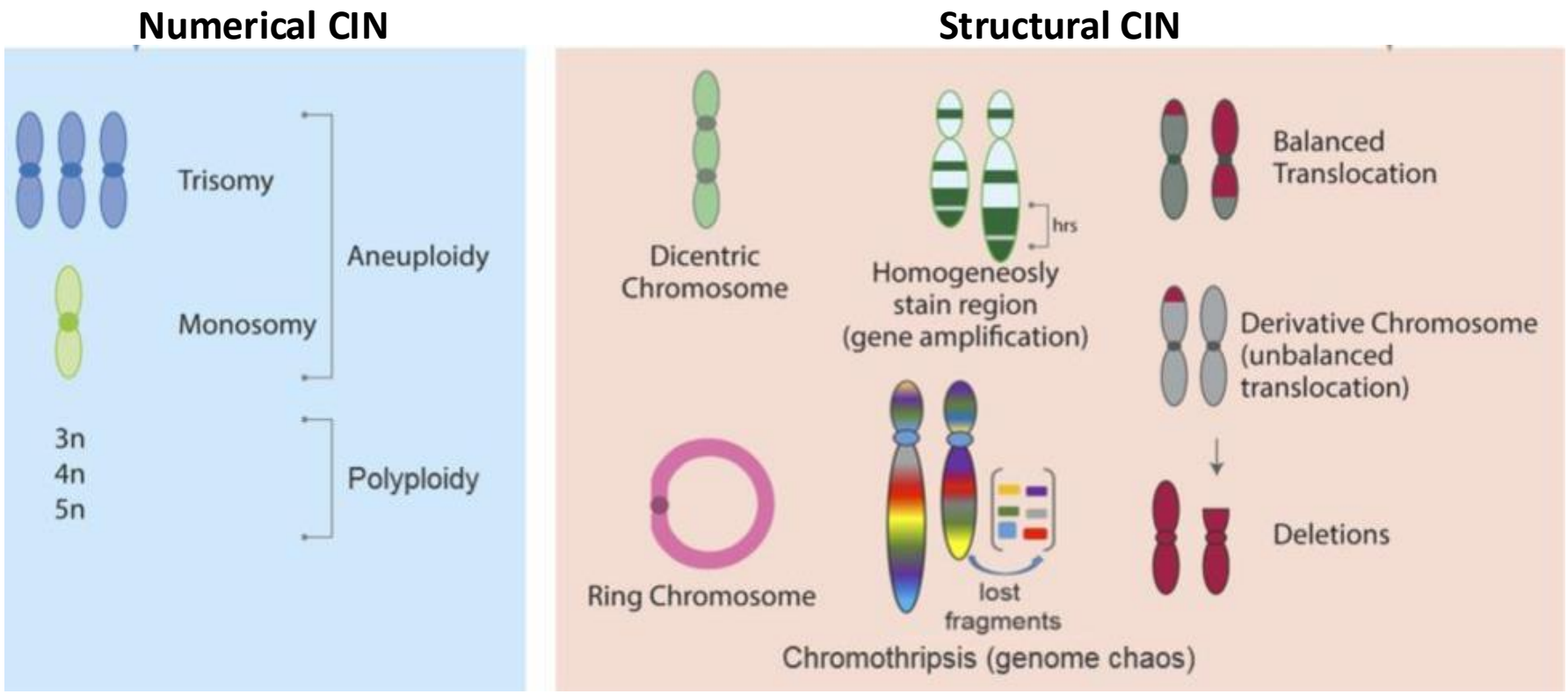
Cunningham et al., 2019

What causes genomic instability?



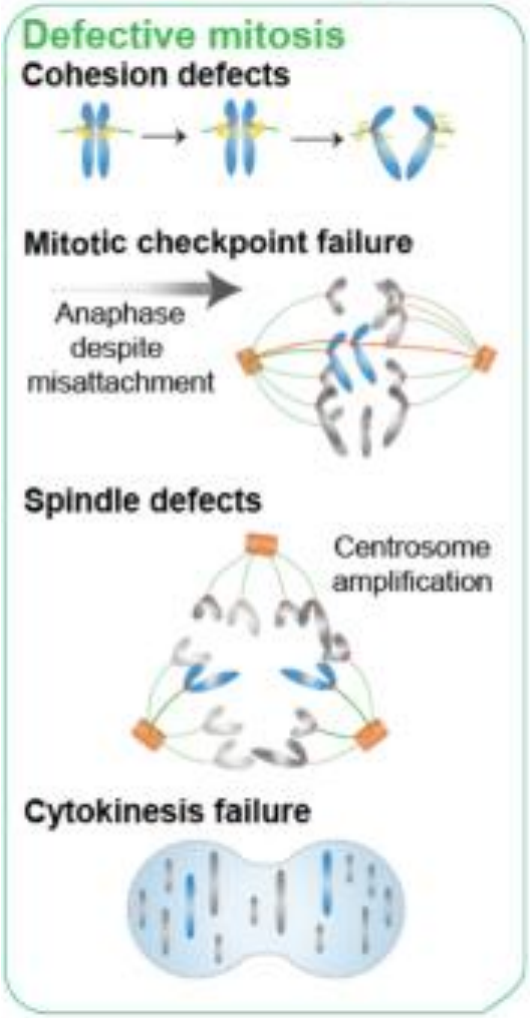
Cunningham et al., 2019

What are some features of CIN?



Vargas-Rondon et al., 2017

Sources of CIN



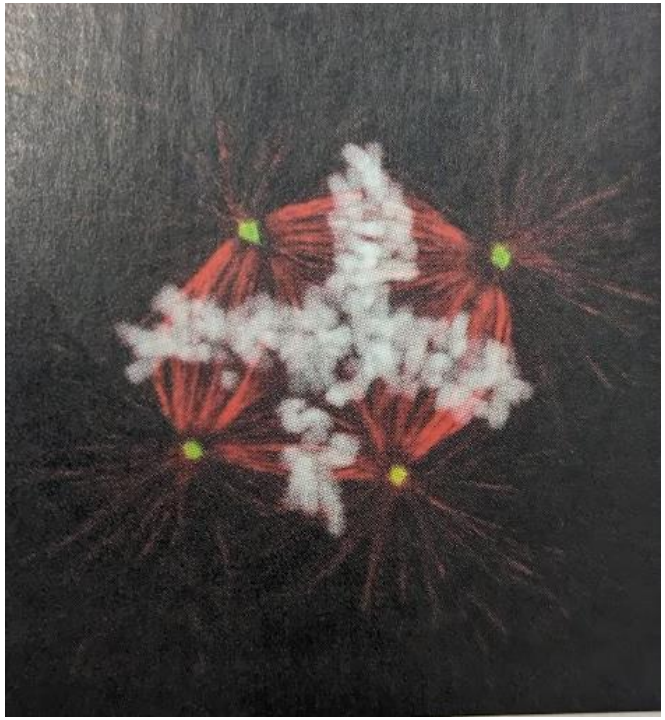
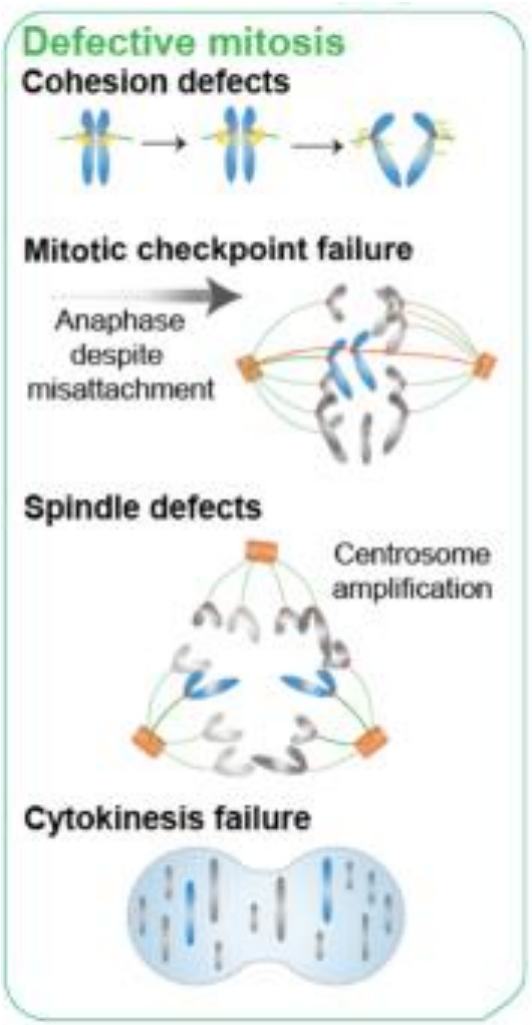
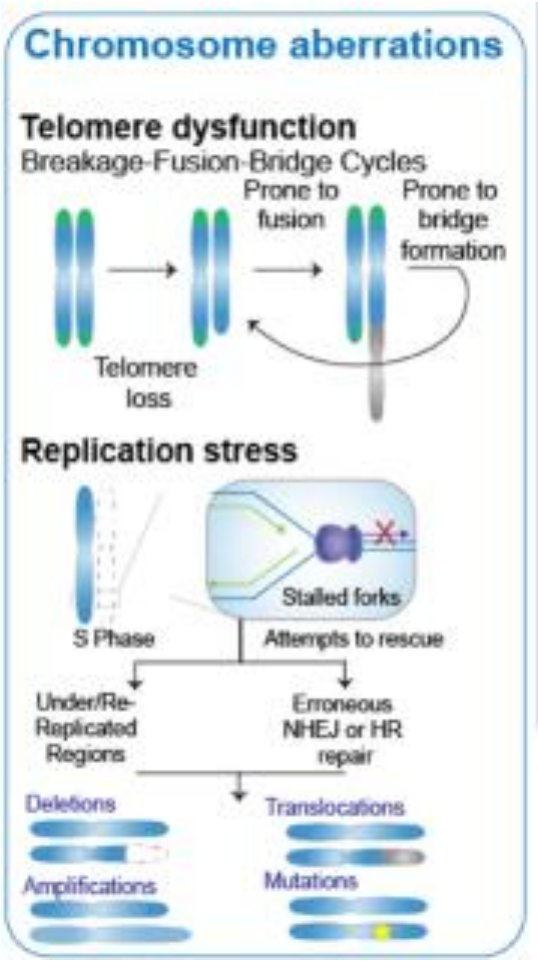


Figure 12.39B – Multipolar mitotic appartus

Tijhuis et al., 2019

Sources of CIN





Tijhuis et al., 2019

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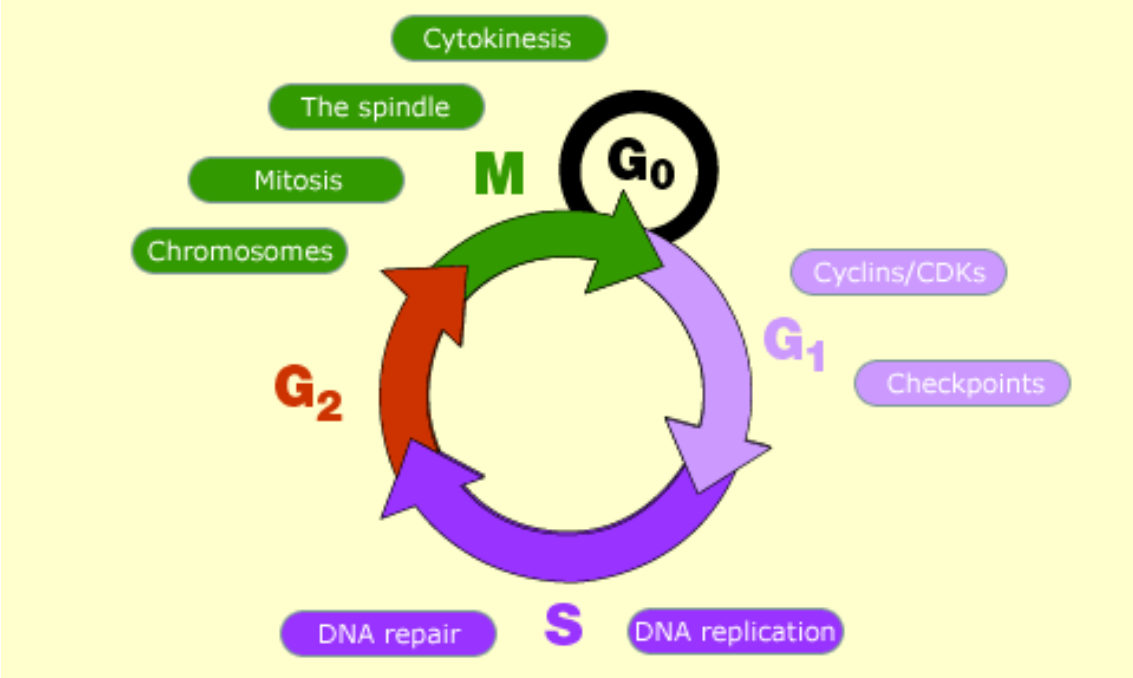
What does the cell do to prevent CIN?

- Closely regulates cell cycle progression
- Checkpoints along the way



Stepping through the cell cycle

- G1 – preparation for S
- Triggering DNA replication – entry into S
- Entering mitosis
- Progressing through anaphase

Stepping through the cell cycle

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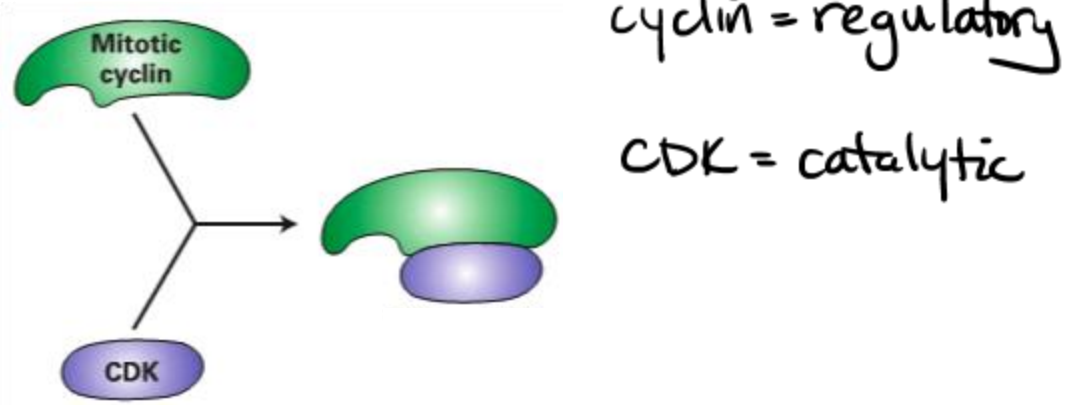
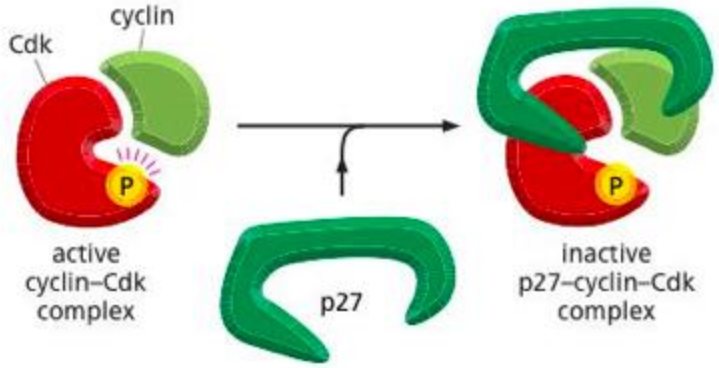
Cyclin and Cyclin Dependent Kinases (CDKs)

- Most basic regulation of the cell cycle is through regulation of cyclin/CDKs
- Form a heterodimer
- Kinase activity of CDK requires cyclin binding partner



Cyclin Dependent Kinase Inhibitors (CKIs)



- Bind to and inhibit phosphorylation by blocking active site
- Must be degraded before next stage can begin

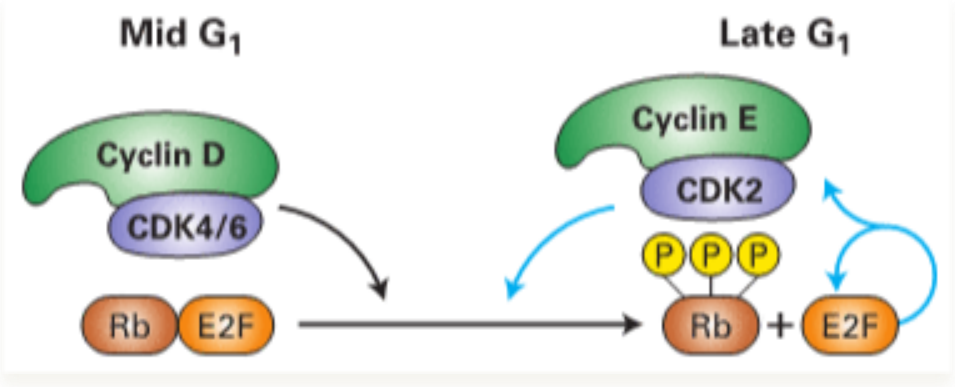
Regulation of Cell Cycle: E2F

- E2F factors are transcription factors that:
  - activate genes involved in DNA synthesis
  - G1/S cyclins, S-phase cyclins, and S-phase CDK, as well as their own expression

Regulation of Cell Cycle: Rb and E2F

- When E2F is bound to Rb, E2F cannot function as a transcriptional activator
- Phosphorylation of Rb by G1 cyclin/CDK at multiple sites prevents it from associating with E2F
- When enough G1/S cyclin has been made, it further phosphorylates Rb and promotes passage through the restriction point

Mid G1 = “g1”
Late G1 = “g1/s cyclins”



Direct inactivation of Rb in tumors

- Rb gene deletion (occurs in retinoblastoma)
- point mutations in the Rb pocket (in retinoblastoma)
- occupancy of the Rb pocket by early proteins of DNA tumor viruses
  - human papilloma virus (HPV), the main etiological agent of human cervical carcinomas
  - HPV encodes two proteins required for tumorigenesis
  - E7 binds the pocket of hypophosphorylated Rb
  - Deregulation of E2F (and the G1/S transition)

From Hee Won Yang's lecture

Indirect inactivation of Rb in tumors

- overexpression of cyclin D1
  - breast cancer, B cell lymphoma
- loss of p16, an inhibitor of Cdk4
  - many human cancers
- inherited point mutation in Cdk4 that renders it insensitive to inhibition by p16
  - familial melanoma

» Inactivation of the Rb pathway occurs in many human tumors!

From Hee Won Yang's lecture

Stepping through the cell cycle

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- Progressing through anaphase

S phase - Origins of Replication

- DNA replication is initiated from prereplication complexes assembled at origins during early G_1
- Initiation of DNA replication occurs at each origin, but only once, until a cell proceeds through anaphase
  - **assures that daughter cells contain the proper number of chromosomes per cell**
- Via phosphorylation, S-phase cyclin-CDK complexes simultaneously trigger initiation from prereplication complexes and inhibit assembly of new prereplication complexes

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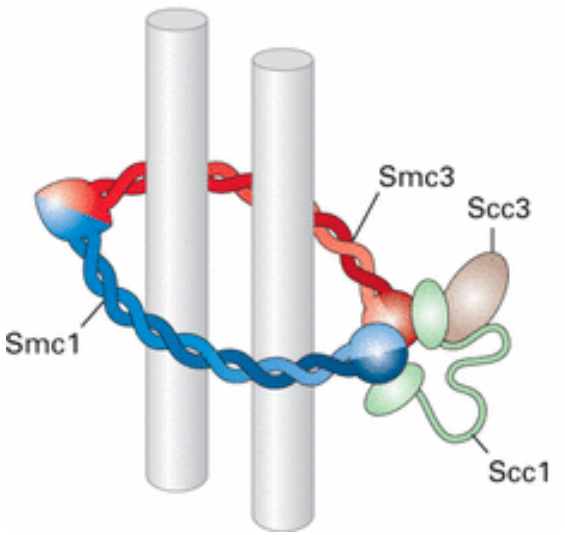
Are any genes in this checkpoint mutated in cancer?

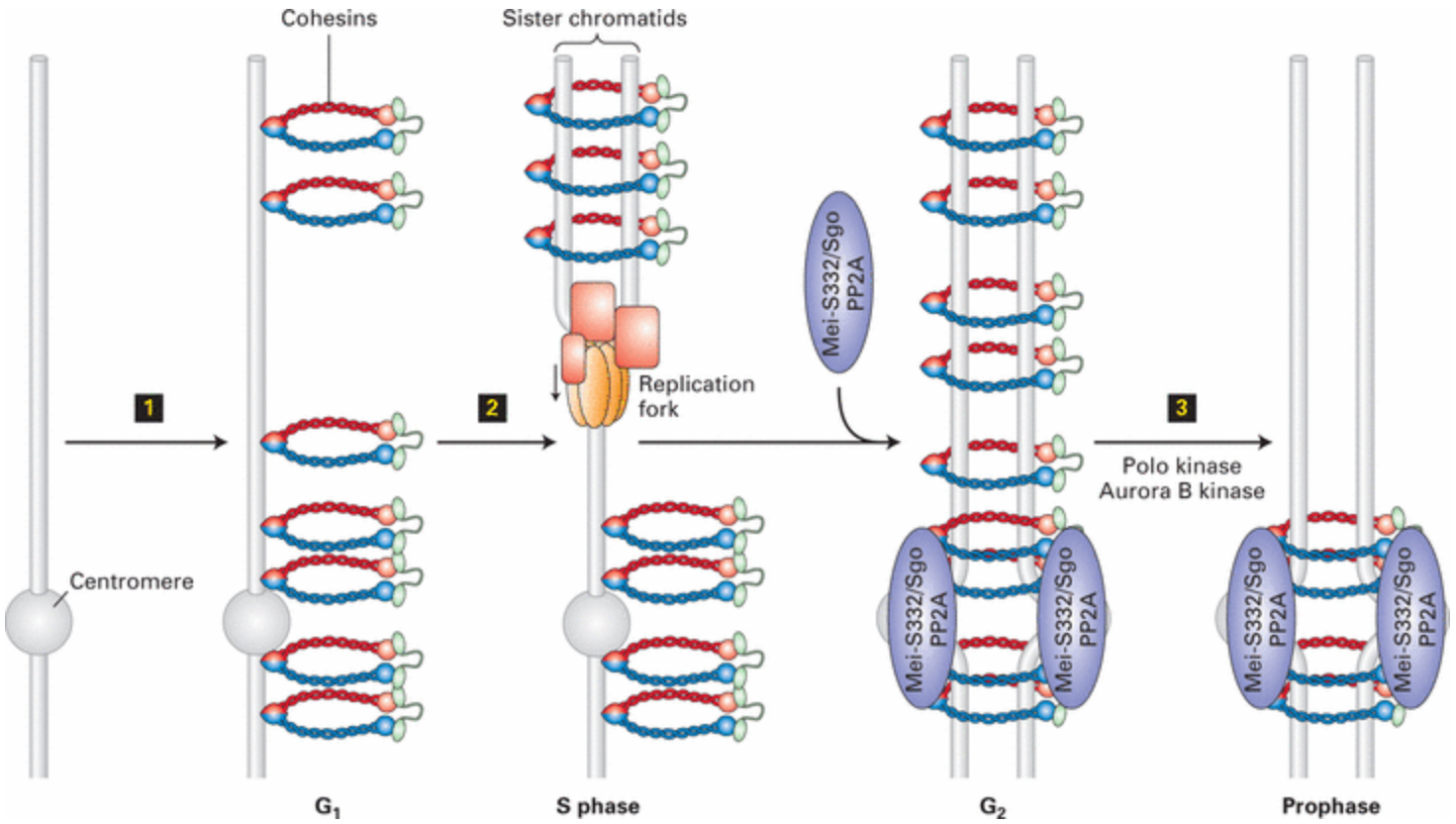
- *POLE* – polymerase ε, mutations result in microsatellite instability
- *CDKN1B* – mutated in a few cancer types
- *CCNE1* – G1/S cyclin, often amplified

Cohesins

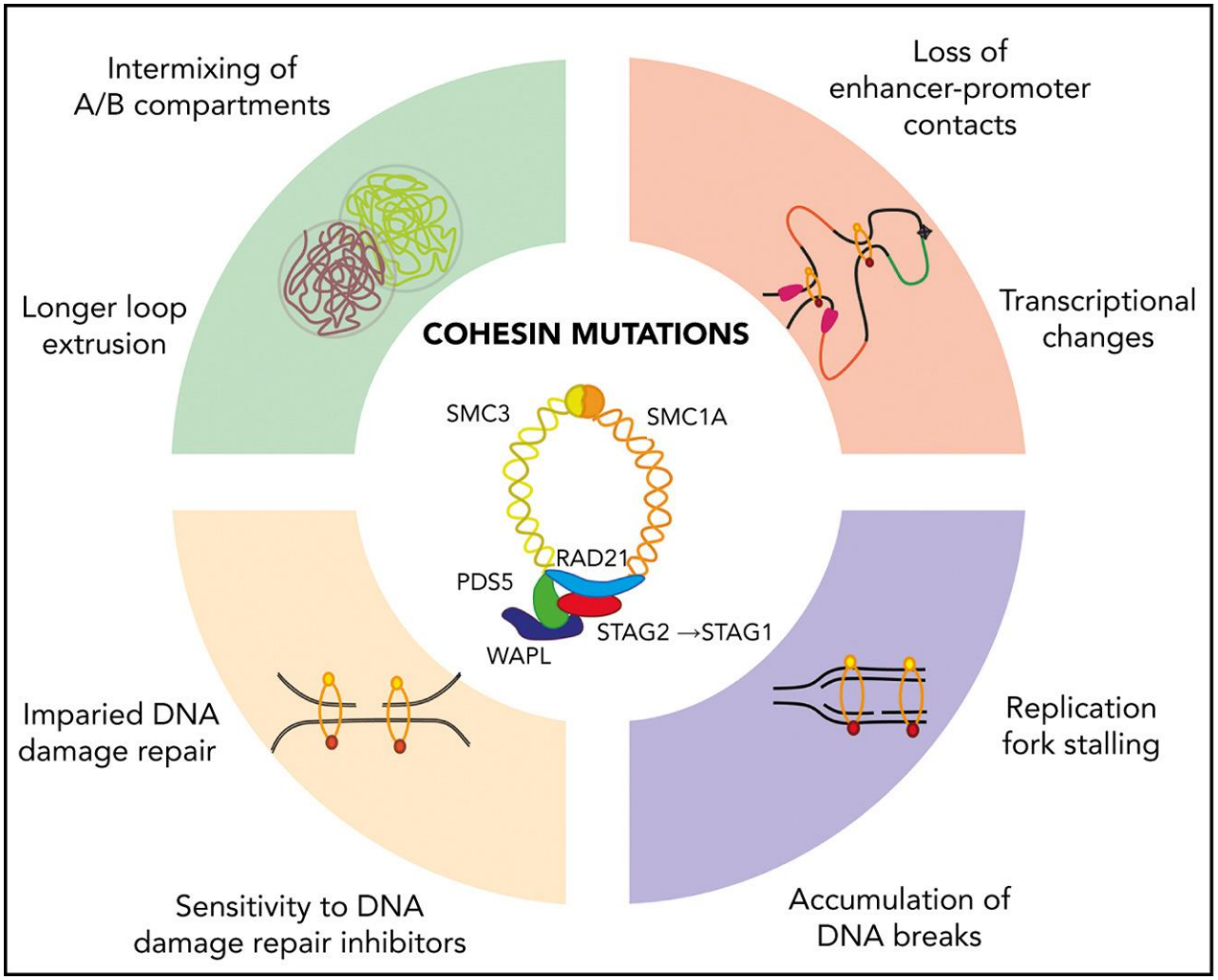


- Complex of four proteins
- Hold sister chromatids together upon replication
- Cleavage induces anaphase and chromosome separation

Cohesins


Cohesins
Sister chromatids
Centromere
1
2
Replication fork
Mei-S332/Sgo PP2A
3
Polo kinase
Aurora B kinase
Mei-S332/Sgo PP2A
Mei-S332/Sgo PP2A
Mei-S332/Sgo PP2A
Mei-S332/Sgo PP2A
G1
S phase
G2
Prophase

Consequences of cohesin mutations in cancer



Jonn and Tothova, 2021

Stepping through the cell cycle

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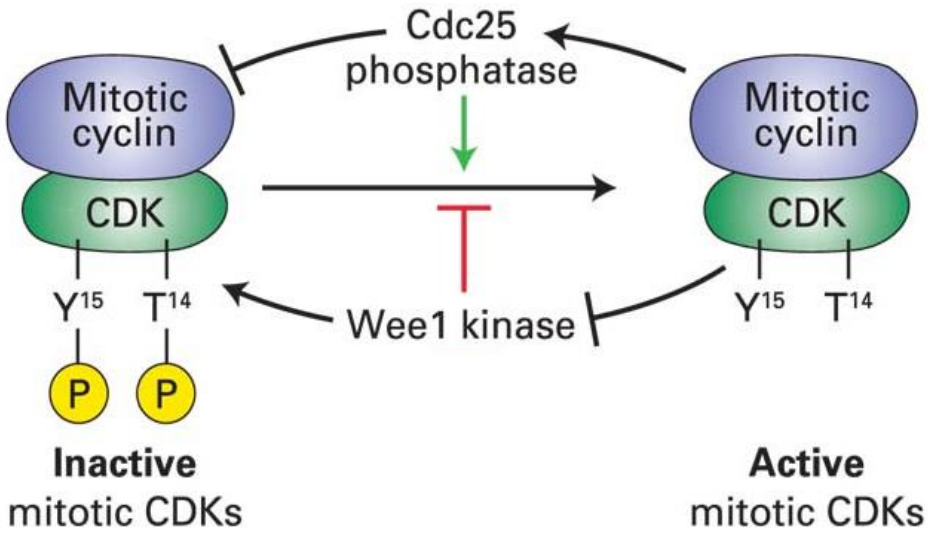
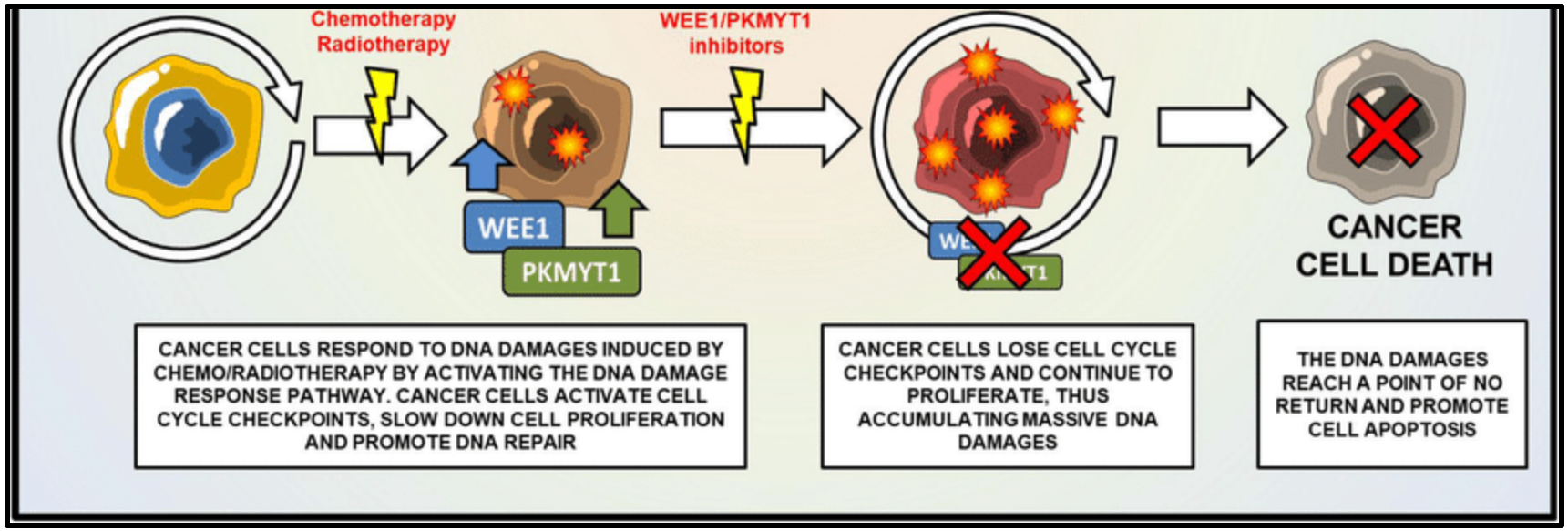
Regulation of Mitotic Cyclin/CDK

- Cyclin and cdk form complex that has low kinase activity
- Wee1 is a protein tyrosine kinase that phosphorylates inhibitory residues in CDK → complex is inactive
- Cdc25 phosphatase removes the inhibitory residue to activate mitotic cyclin (MPF)



Checkpoint and cancer

- *CDC25C* is mutated in FPD/AML patients
- Wee1 inhibitors in the clinic



Ghelli et al., 2020

Stepping through the cell cycle

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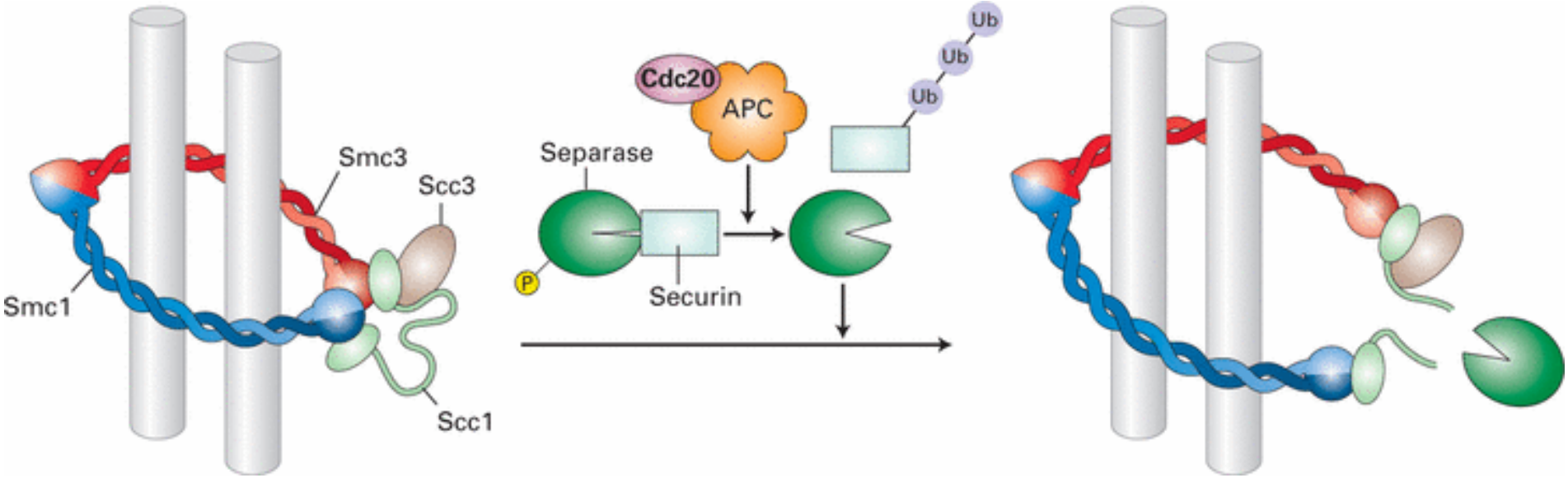
- **Progressing through anaphase**

Cohesion of chromatids at centromere is tightly regulated

Separase: Enzyme that cleaves part of cohesin to release chromosomes prior to separation

Securin: Inhibits separase thus ensuring proper timing of chromosome separation

APC/C and Cdc20: target securin for degradation



Bi-oriented Chromosome Attachment

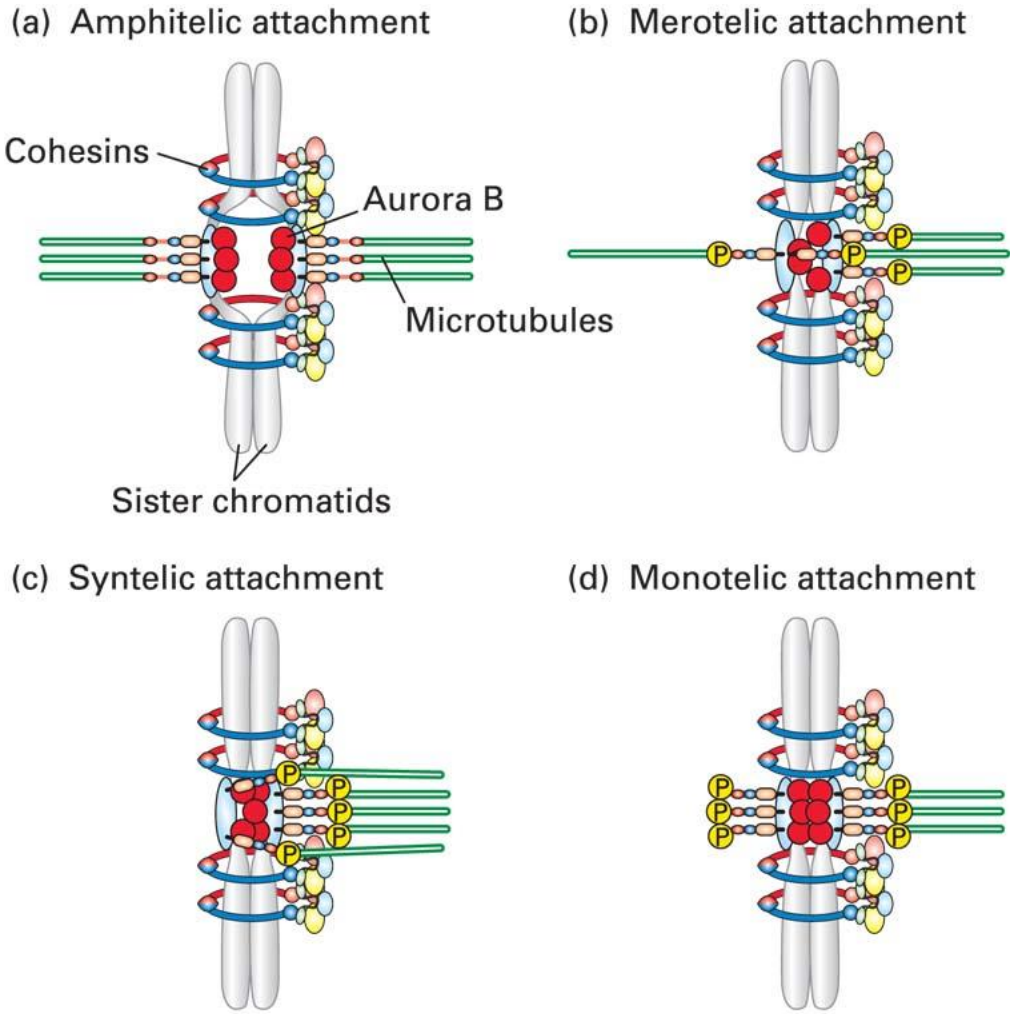


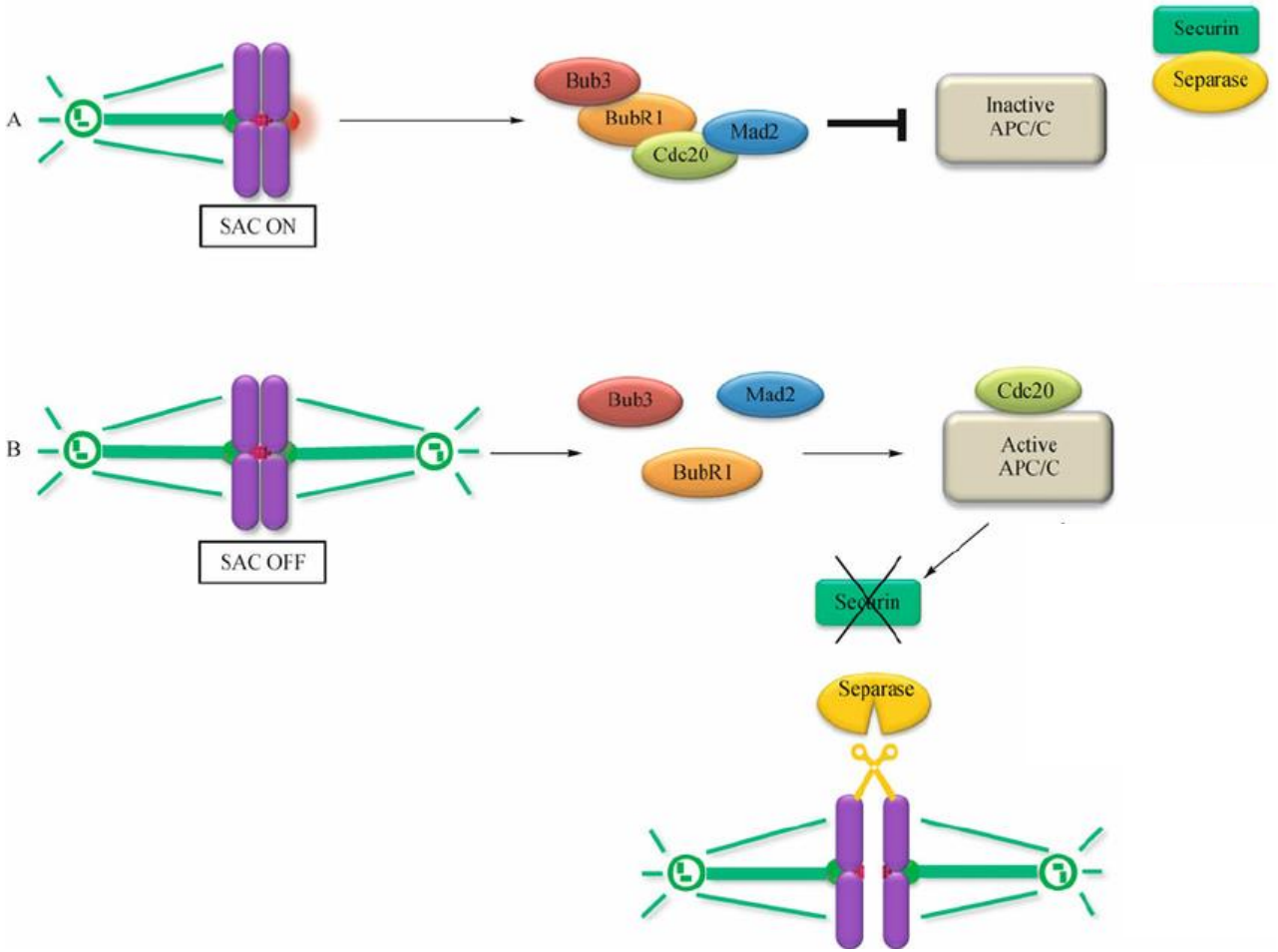
Figure 19.22
Molecular Cell Biology, eighth edition

Correct/incorrect microtubule attachments are sensed by tension by Aurora B

- When incorrect (b-d), it phosphorylates kinetochore components, destabilizing microtubule binding
- When correct (a), can't phosphorylate

Spindle Assembly Checkpoint

Spindle assembly checkpoint prevents entry into anaphase until every kinetochore of every chromatid is properly associated with a microtubule



Adapted from Barbosa et al., 2011

What does the centromere really look like?

Cell

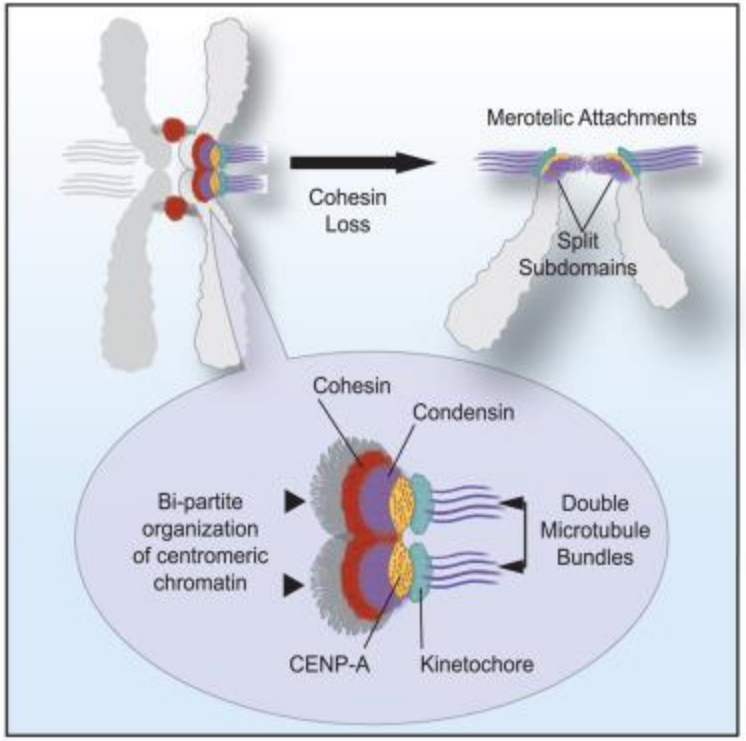
CellPress

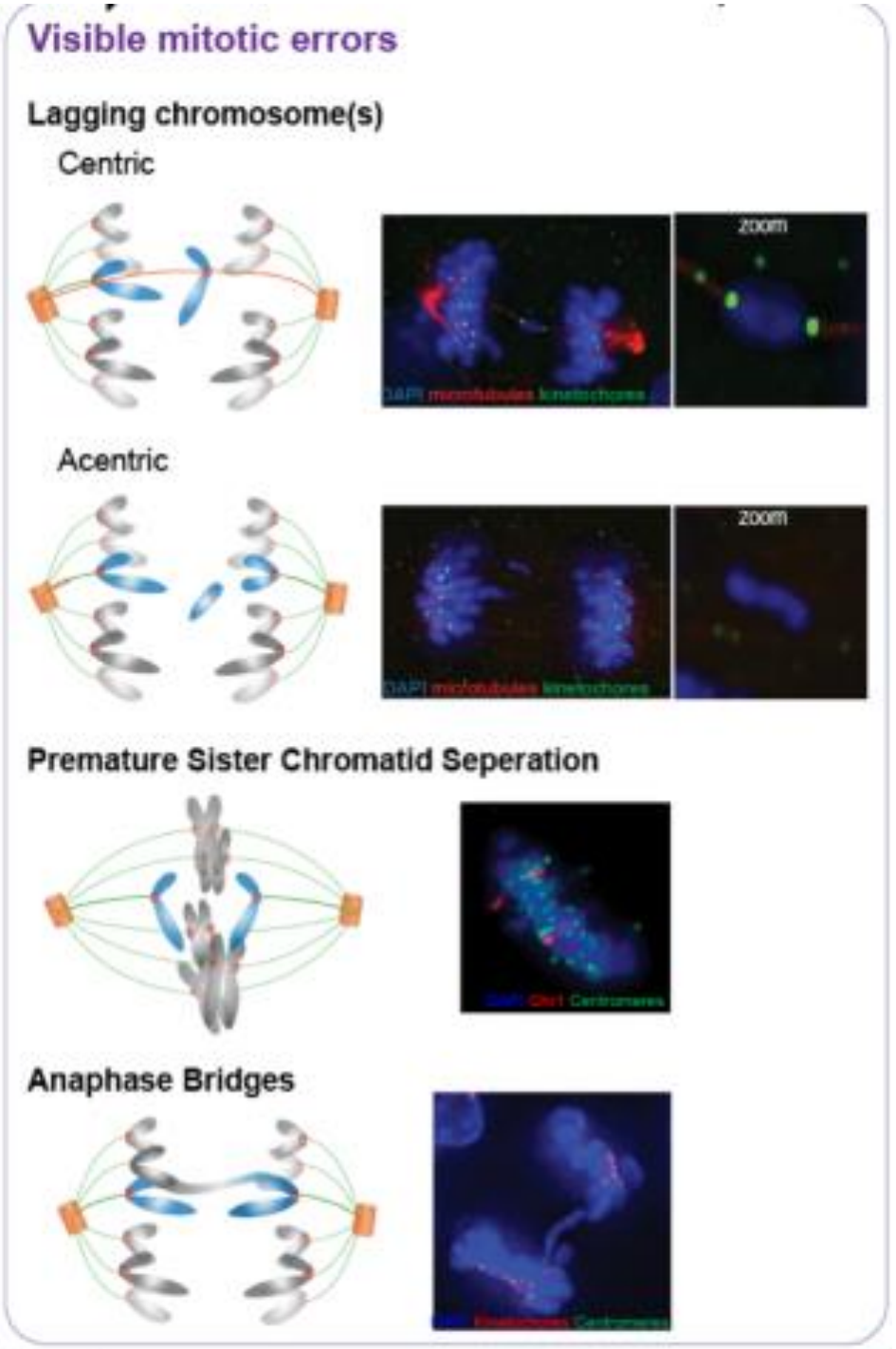
Volume 187, Issue 12, 6 June 2024, Pages 3006-3023.e26

Article

Vertebrate centromeres in mitosis are functionally bipartite structures stabilized by cohesin

Carlos Sacristan [1 10], Kumiko Samejima [2 10], Lorena Andrade Ruiz [1], Moonmoon Deb [2], Maaike L.A. Lambers [1], Adam Buckle [3], Chris A. Brackley [4], Daniel Robertson [2], Tetsuya Hori [5], Shaun Webb [2], Robert Kiewisz [6 7], Tristan Bepler [6], Eloïse van Kwawegen [1], Patrik Risteski [8], Kruno Vukušić [8], Iva M. Tolić [8], Thomas Müller-Reichert [9], Tatsuo Fukagawa [5], Nick Gilbert [3], Davide Marenduzzo [4]...Geert J.P.L. Kops [1 11]





Consequences of failed spindle assembly checkpoint

Tijhuis et al., 2019

What can happen to a lagging chromosome?

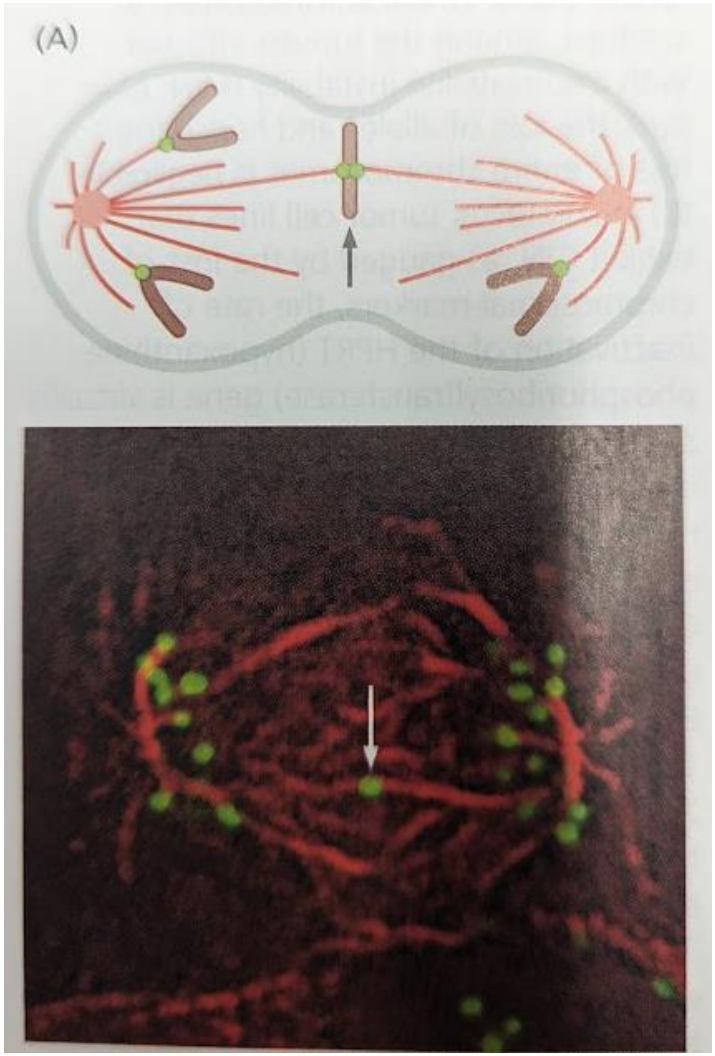
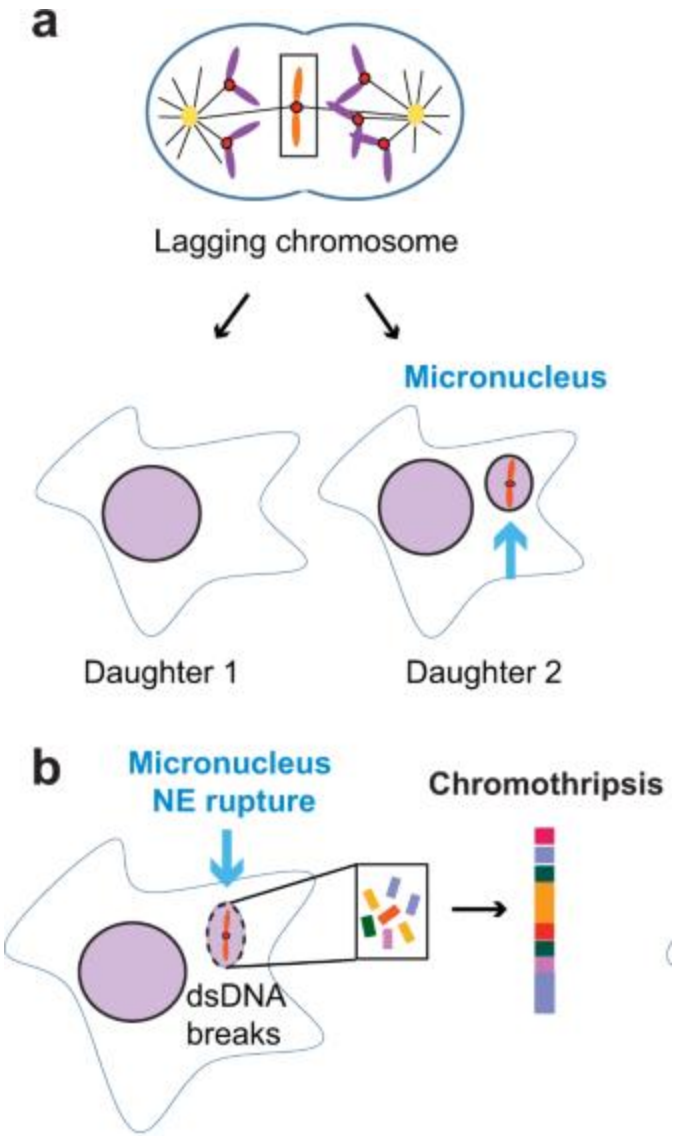


Figure 12.38a

Chromothripsis (chromosome shattering) can occur in micronuclei formed from lagging chromosomes

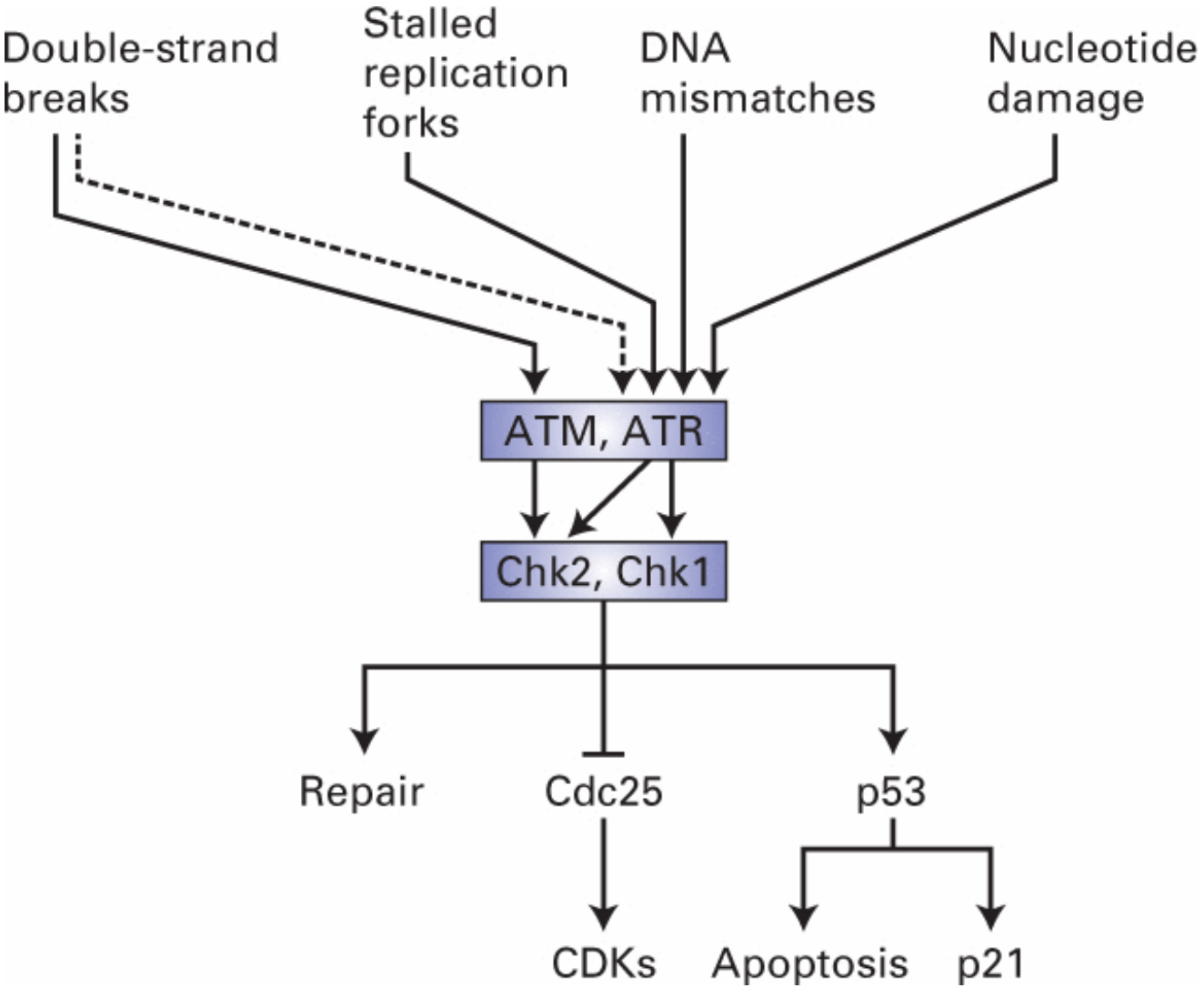


Kwon et al., 2020

DNA damage checkpoints occur throughout the cell cycle

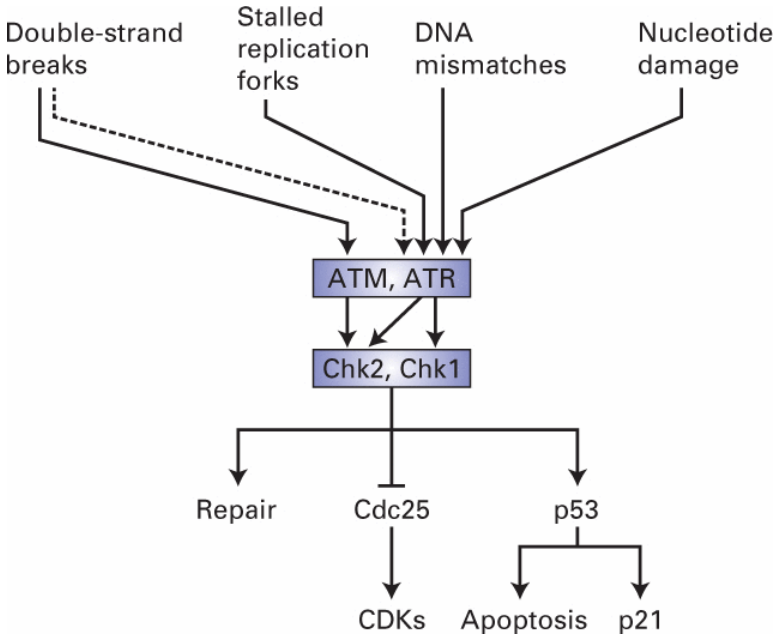
- Arrest in G1 and S prevents copying damaged bases
- Arrest in G2 allows DNA double stranded breaks to be repaired before mitosis

DNA Damage Recognized by ATM/ATR Proteins



DNA Damage Recognized by ATM/ATR Proteins

- <u>Double strand breaks:</u>
  - Proteins signal presence of DSB to ATM kinases
  - ATM then phosphorylates and activates Chk2
  - Chk2 phosphorylates Cdc25A phosphatase, to mark it for degradation
  - Cdc25A cannot then remove inhibitory phosphate on S-phase CDKs
  - **Degradation of Cdc25A results in cell cycle arrest in G1 or S**



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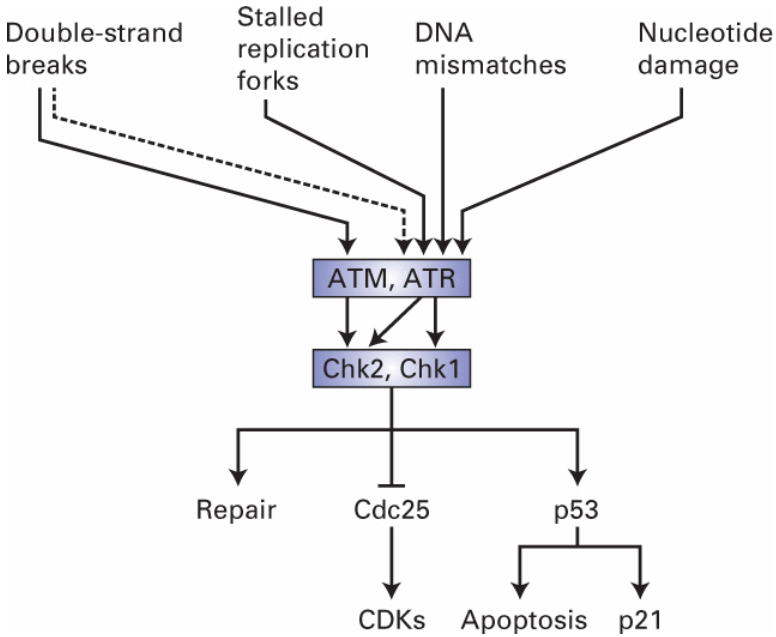
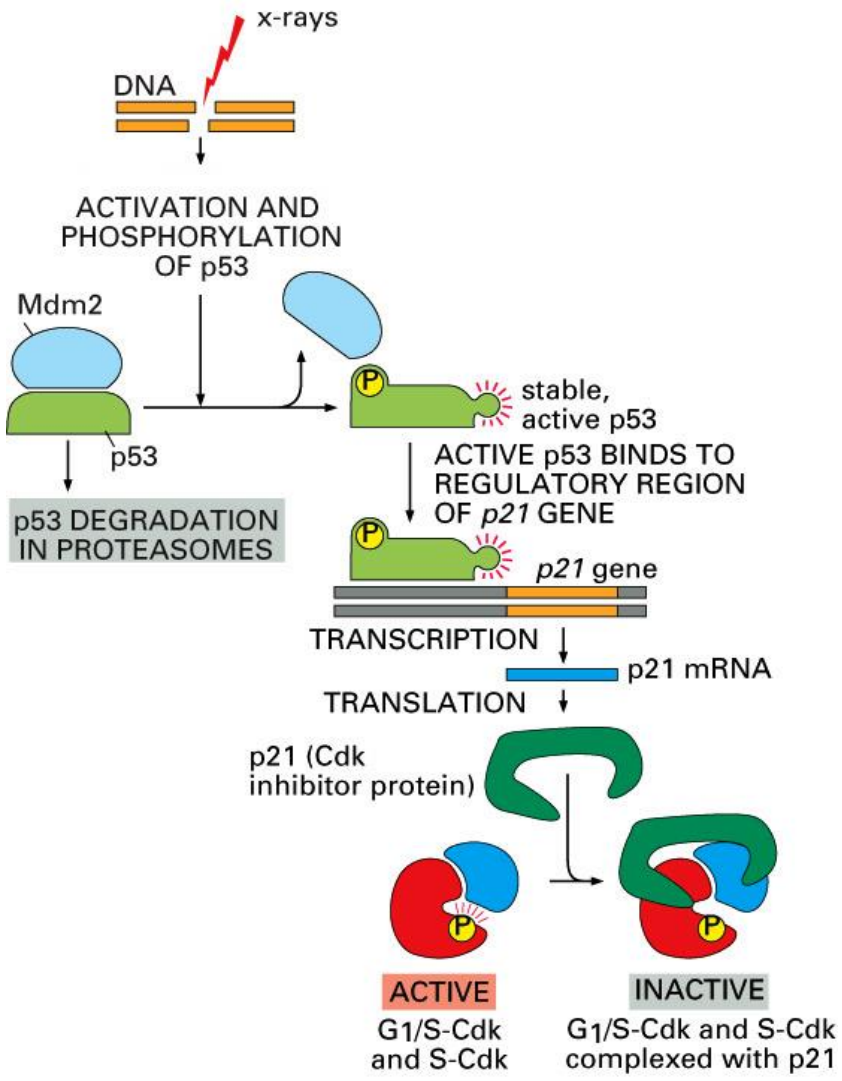
- ATR and Chk1 in response to gamma-irradiation:
  - phosphorylate Cdc25A
  - Chk1 also inactivates Cdc25C preventing activation of CDK1 (mitotic CDK)



p53 is a key regulator of DNA damage response



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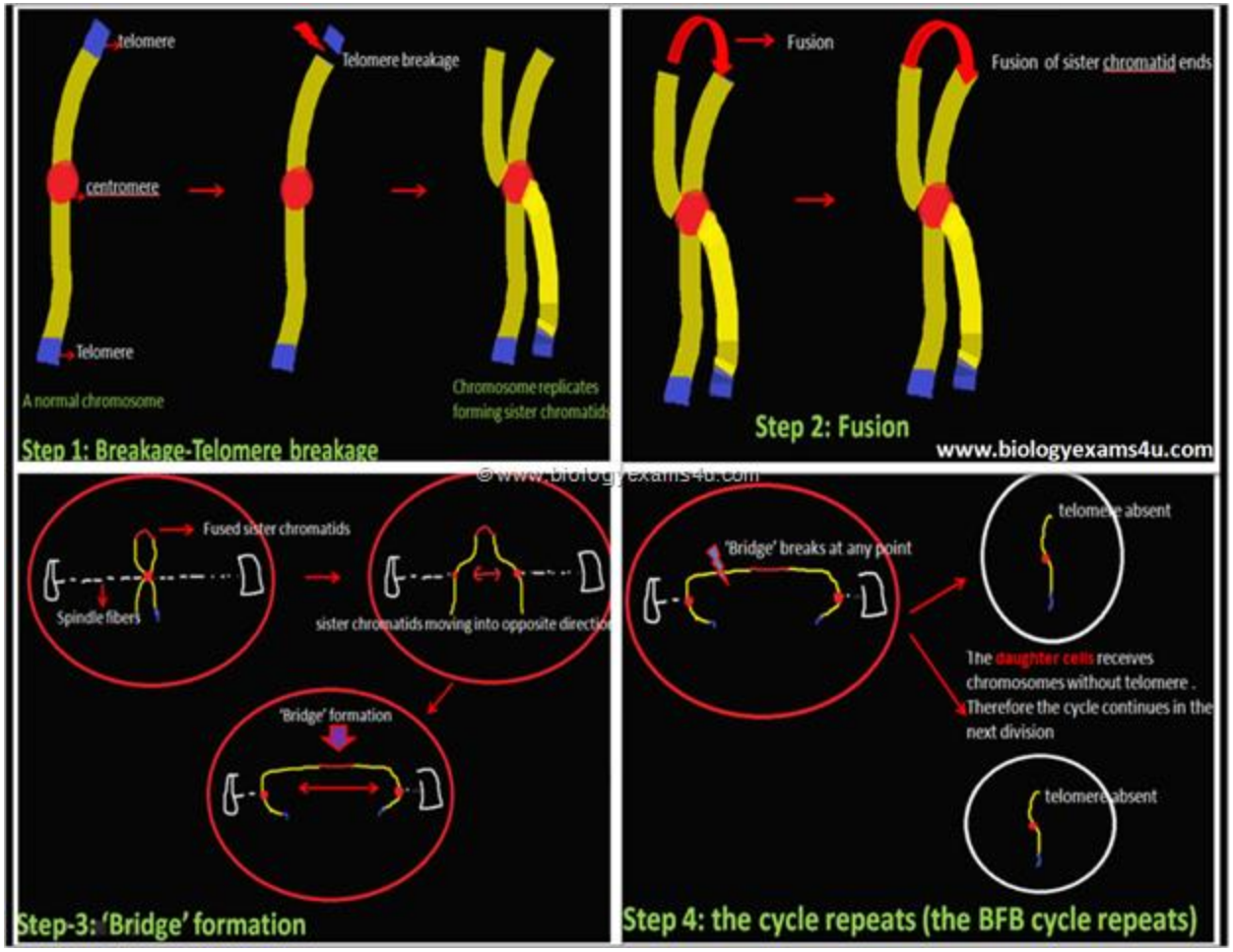
Breakage-fusion bridge cycle



https://www.biologyexams4u.com/2013/10/what-is-breakage-fusion-bridge-bfb.html

Fusions are commonly observed in cancer

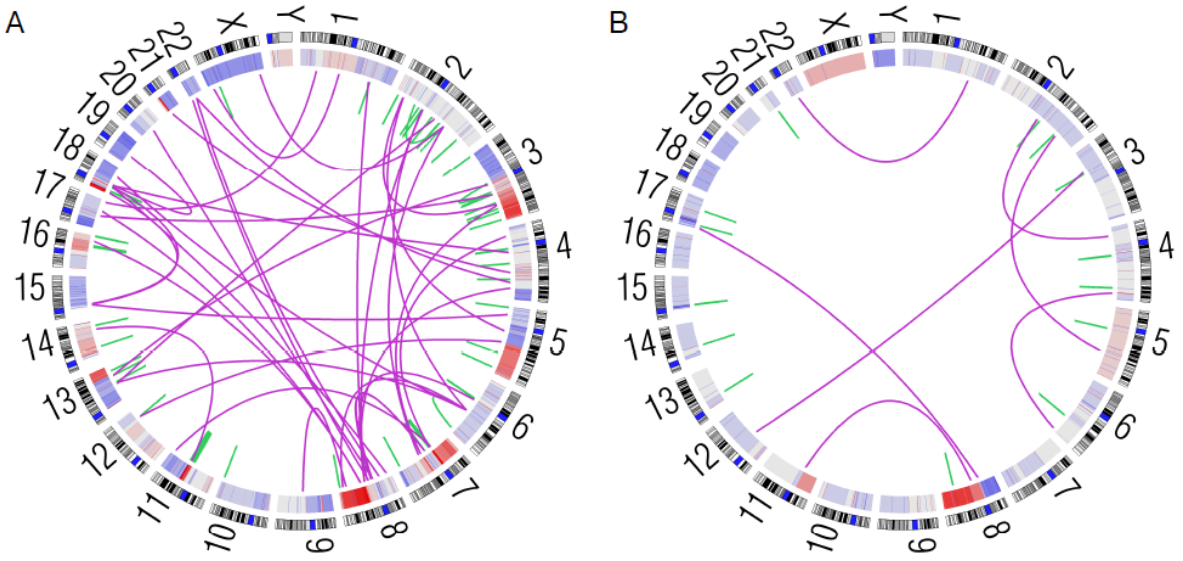
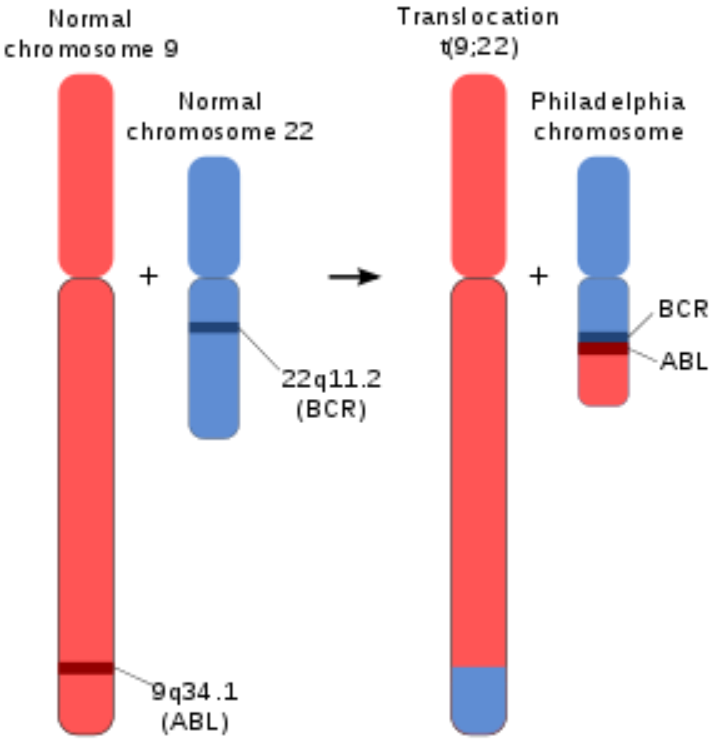
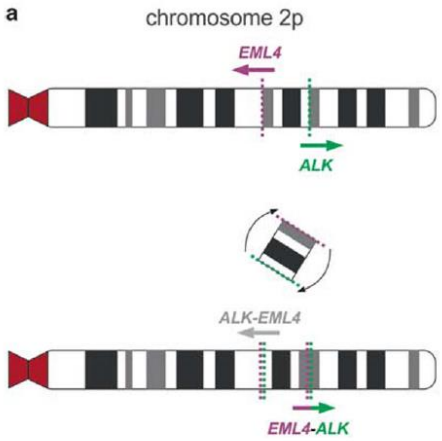


Figure 12.34

Figure S5. Circos plots derived from whole genome sequencing. An oropharyngeal tumor (62699, A) and a hypopharyngeal tumor (62469, B) are shown. Purple lines represent interchromosomal rearrangements. Green lines represent intrachromosomal rearrangements. Each chromosome is delineated by the appropriate letter or number outside of the plot, as well as the corresponding segments of the concentric circles. The segments of the outer circle represent the normal banding pattern of each chromosome, with blue indicating the centromere. The segments of the inner circle represent copy number changes of each chromosome, inferred from SNP array data. Red indicates copy number gain, blue indicates copy number loss, and the intensity of the color correlates with the magnitude of gain or loss.

Fusions can result in oncogenic translocations





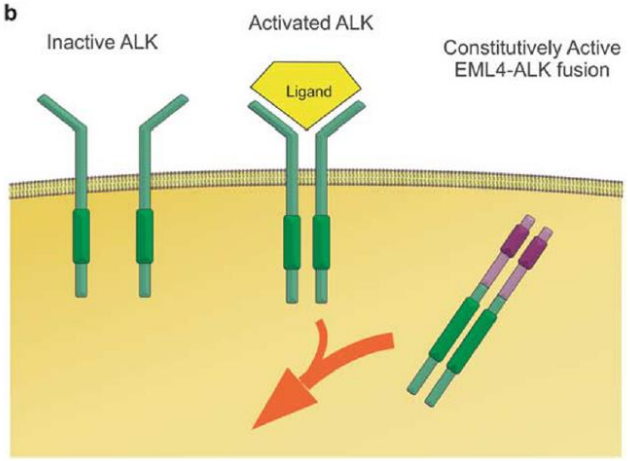


Figure 12.33

In-Class Question

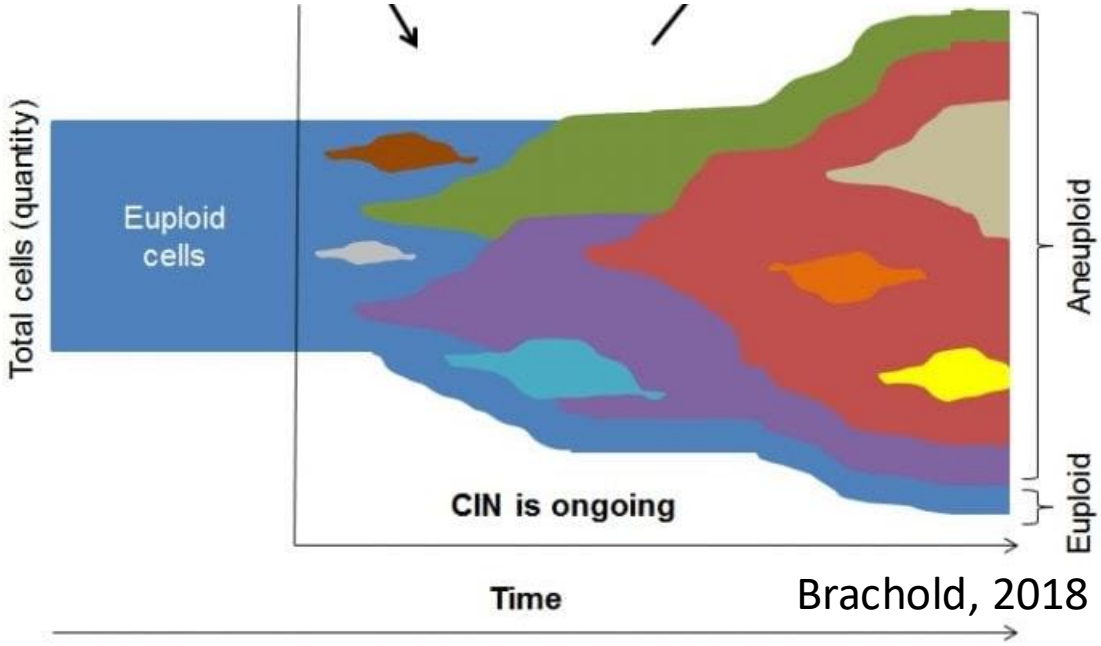
What is the difference between **aneuploidy** and **chromosome instability**?

A) There is no difference, both are changes in chromosome number.

B) Chromosome instability is the ongoing change in chromosomes, whereas aneuploidy is the state of incorrect chromosome number.

C) Chromosome instability is the state of incorrect chromosome number, whereas aneuploidy is the ongoing change in chromosomes.

Chromosome instability (CIN) is ongoing changes in chromosomes



Brachold, 2018

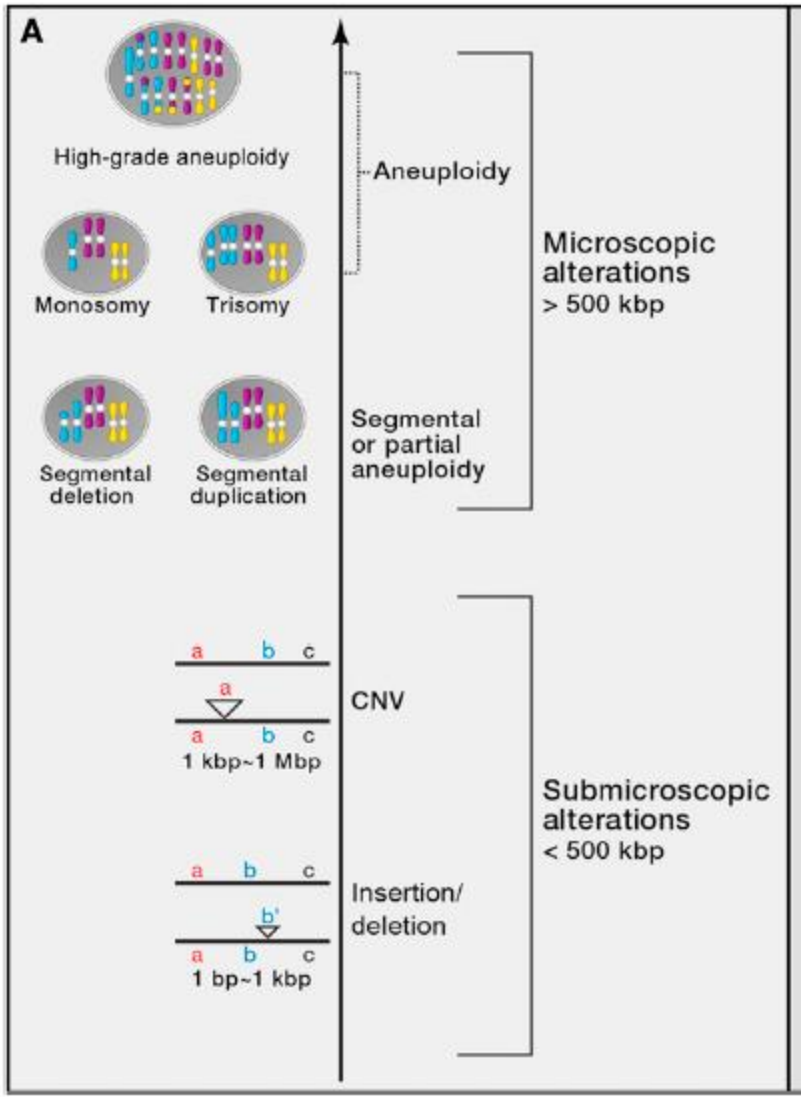
Measuring CIN in patient samples is quite challenging

Aneuploidy Definitions

Polyploidy = "balanced genomic state", includes diploid, tetraploid, octoploid, etc.

Aneuploidy = "a state in which the cell does not contain an exact multiple of the haploid chromosome complement"

- Stable aneuploidy
- Result from chromosomal instability (CIN), specific gains/losses change over time



Note: Unchanged stoichiometry does NOT mean aneuploidy

Tang and Amon, 2013

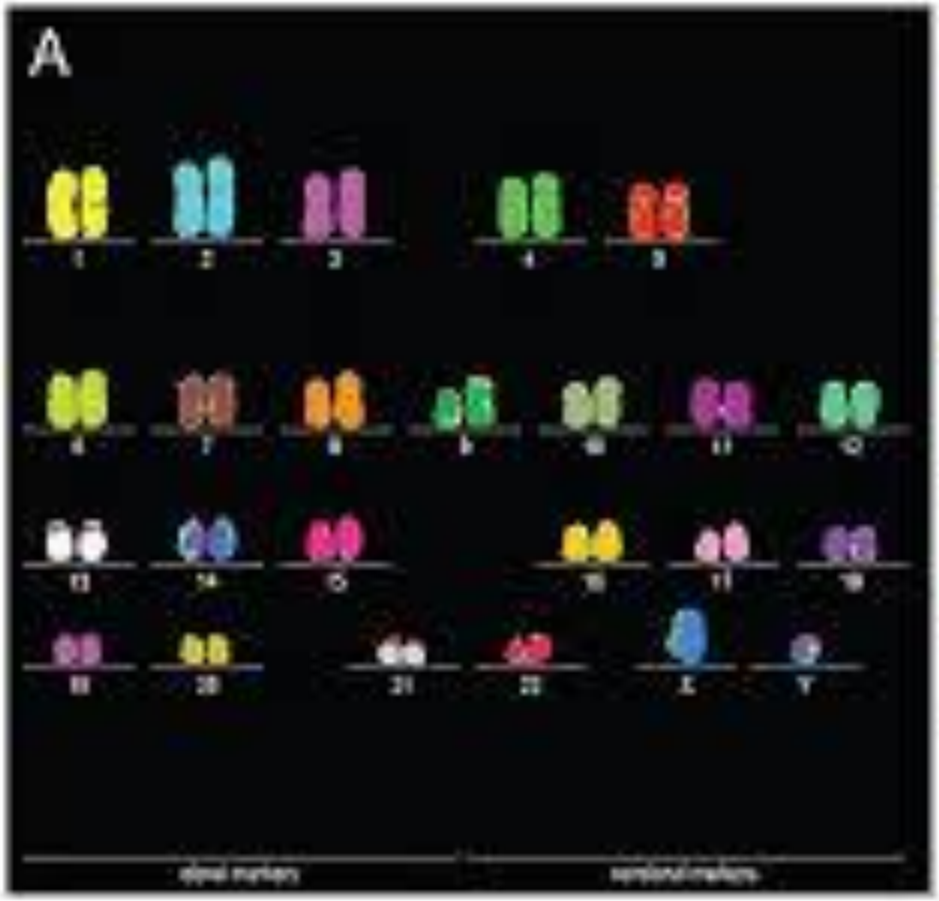
In-Class Question

Are cells with a translocation aneuploidy?

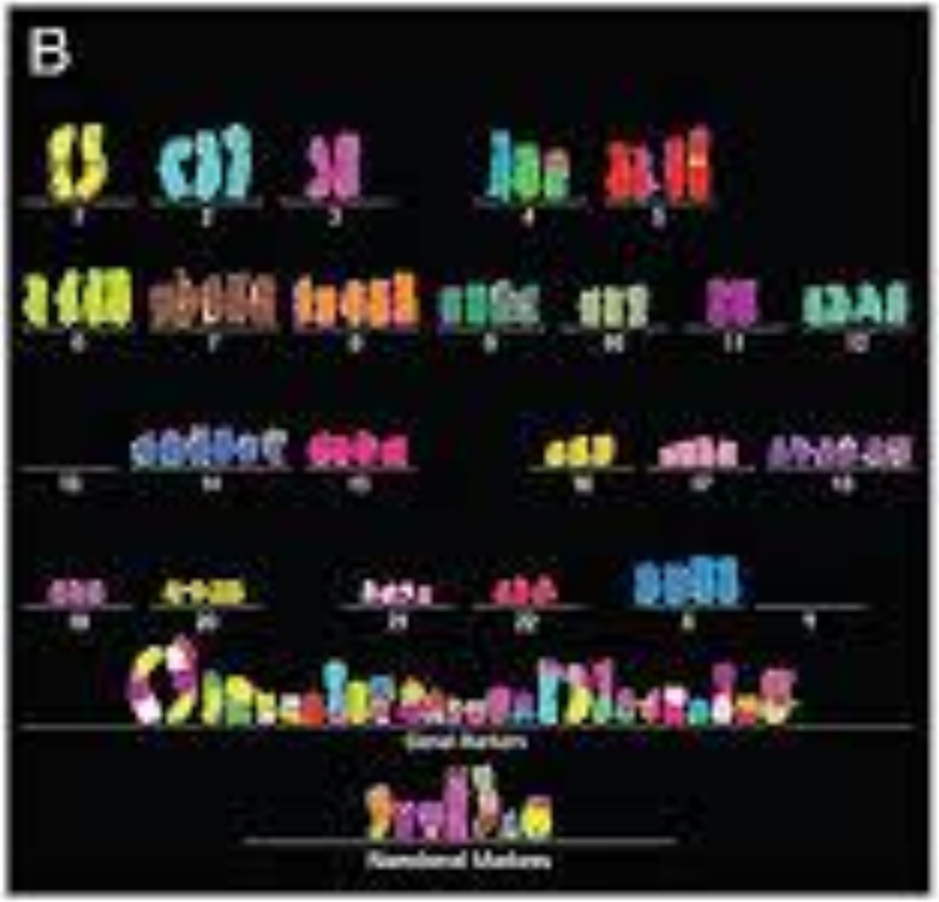
- A) Yes
- B) No
- C) It depends.

Spectral karyotyping demonstrates aneuploidy in cancer

Normal Cell

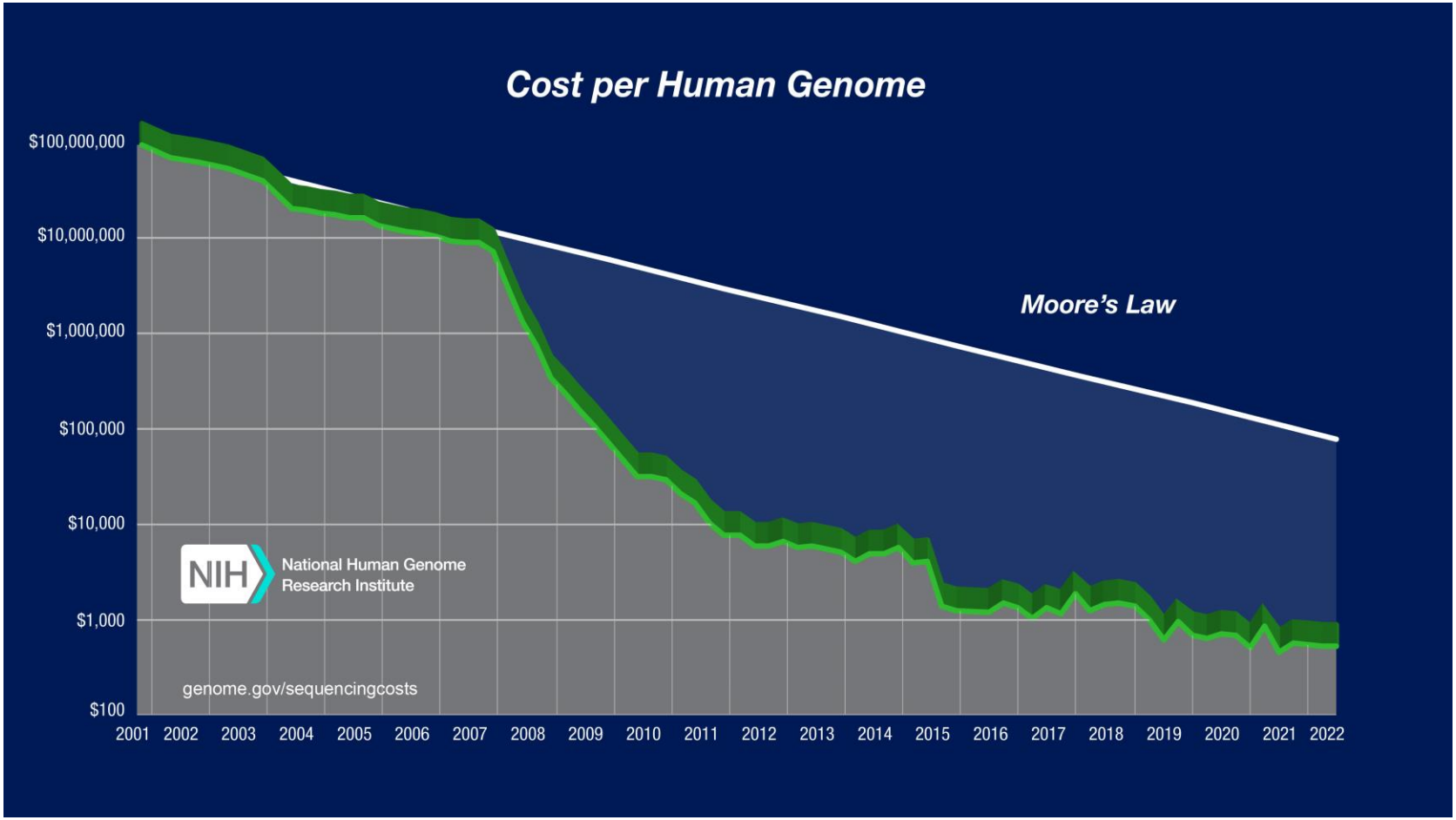


Cancer Cell



Source: UC Berkeley News

Sequencing Advances



Matches figure 12.40

National and International Efforts

The Cancer Genome Atlas

Over 10,000 tumors collected from 33 cancer types (in the US)

International Cancer Genome Consortium

Over 20,000 tumors collected from 22 cancer types (internationally)

AACR American Association for Cancer Research FINDING CURES TOGETHER™

PROJECT GENIE Genomics Evidence Neoplasia Information Exchange

Over 130,000 tumors from ~10 institutions in the US/Canada (including Columbia)

Where to get this data?

The Cancer Genome Atlas (TCGA):

- Cbioportal (user interface)
- Websites to download data (including: https://gdc.cancer.gov/about-data/publications/pancanatlas)
- Data available: 10,000+ cancers with mutation, copy number, gene expression, and some clinical data

AACR GENIE

- Cbioportal (user interface)
- Sage bionetworks (get access for more unprocessed data)
- Data available: 200,000+ samples with clinical and mostly WES (mutation) data from wide number of institutions

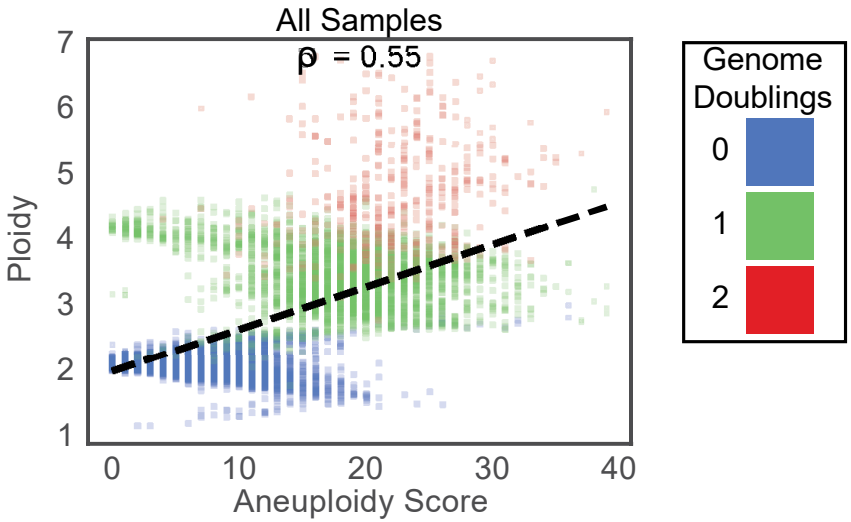
Cancer cell line data in "dependency map"

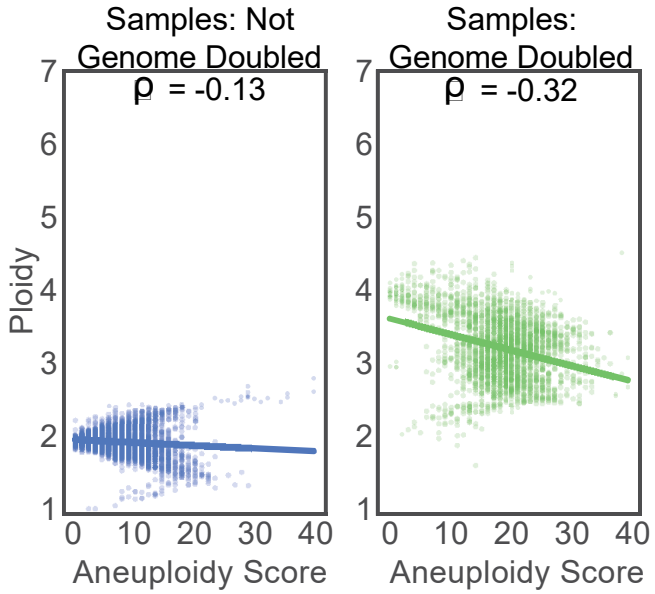
- User interface (depmap.org/portal)
- Downloadable data (same website)
- Data available: Mutation, copy number, gene expression, CRISPR and chemical screening

GenePattern as a place to analyze data – NO CODING req'd

- Differential gene expression, pathway analysis & more!

Aneuploidy correlations with DNA content

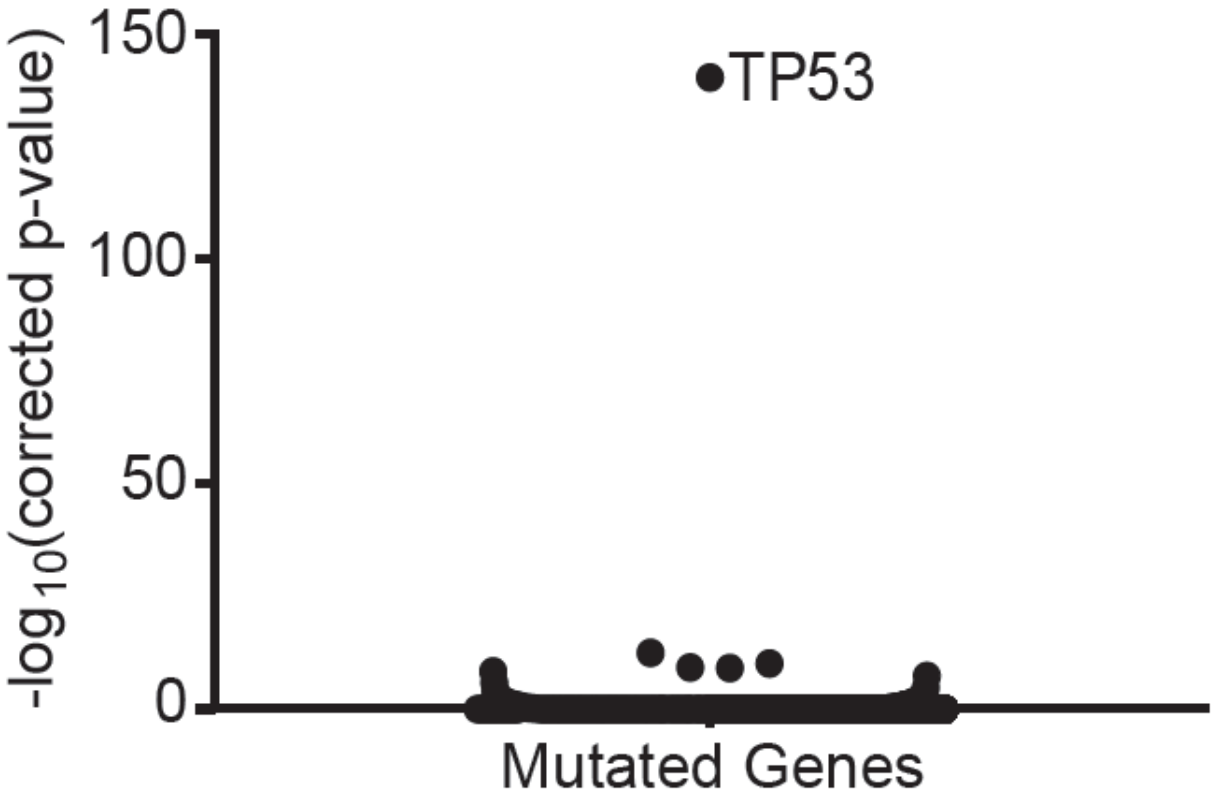




- Tumors that have undergone genome doubling have higher levels of aneuploidy
- Aneuploidy negatively correlates with ploidy, demonstrating that “loss” events are more common than “gain” events

Taylor et al., 2018 *Cancer Cell*

Mutations in TP53 Correlate with Aneuploidy



MIN vs CIN

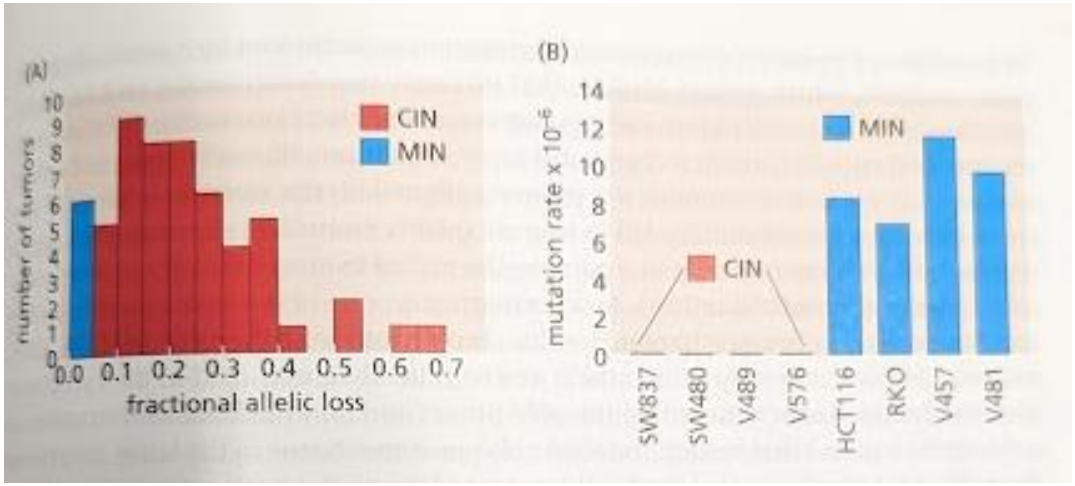
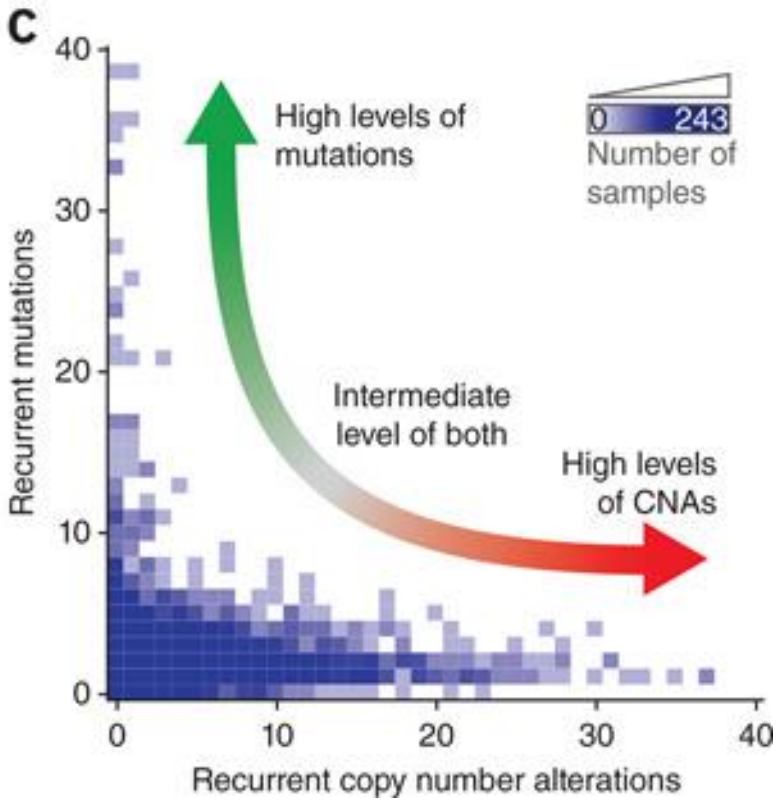


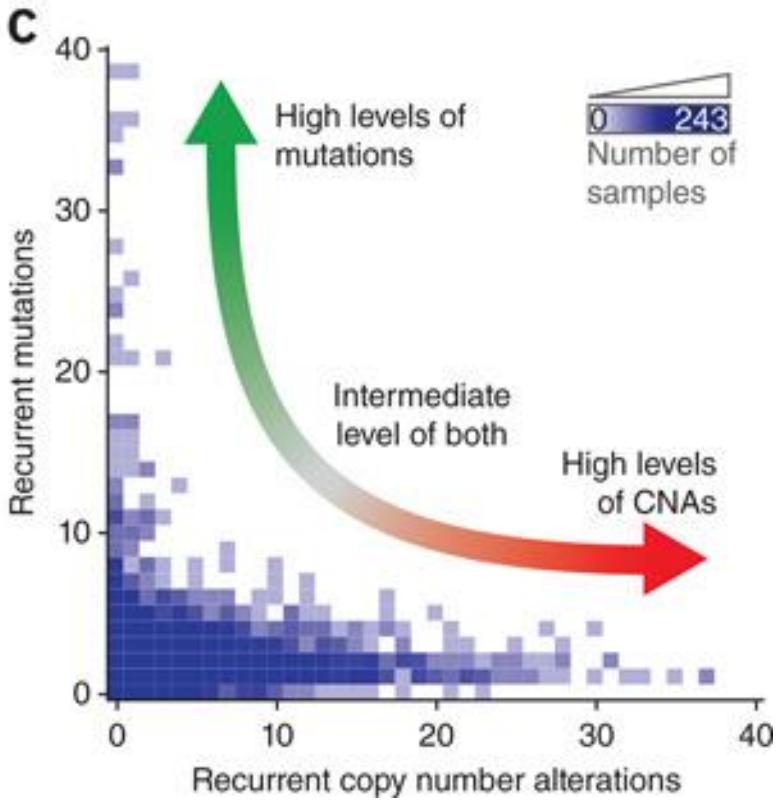
Figure 12.37

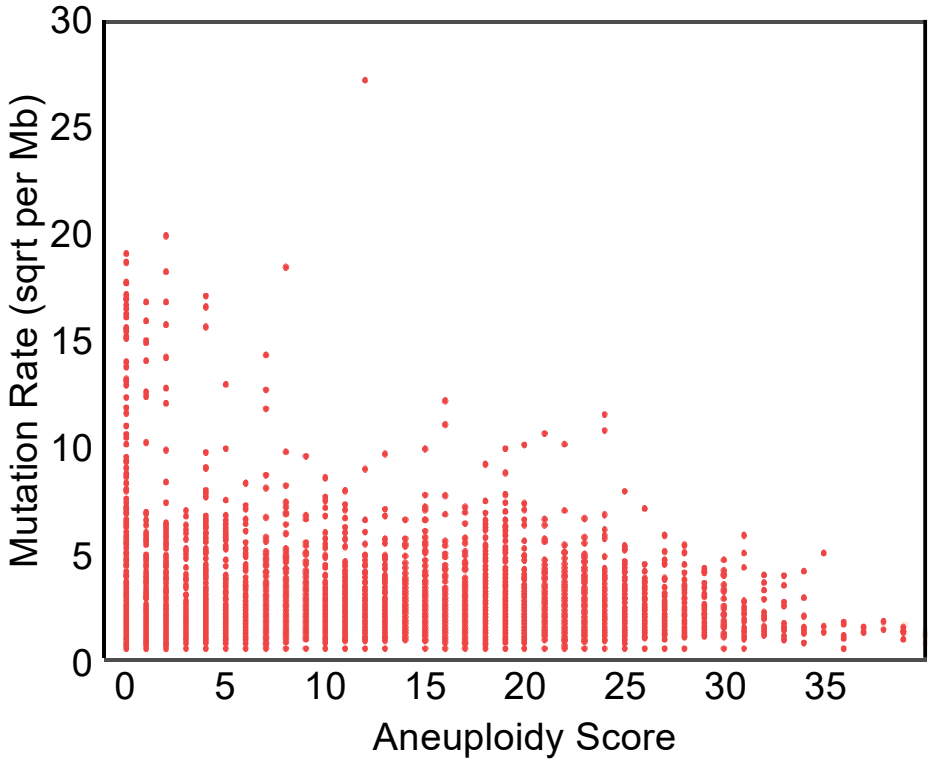
Does mutation rate correlate with aneuploidy?



Ciriello et al., 2013

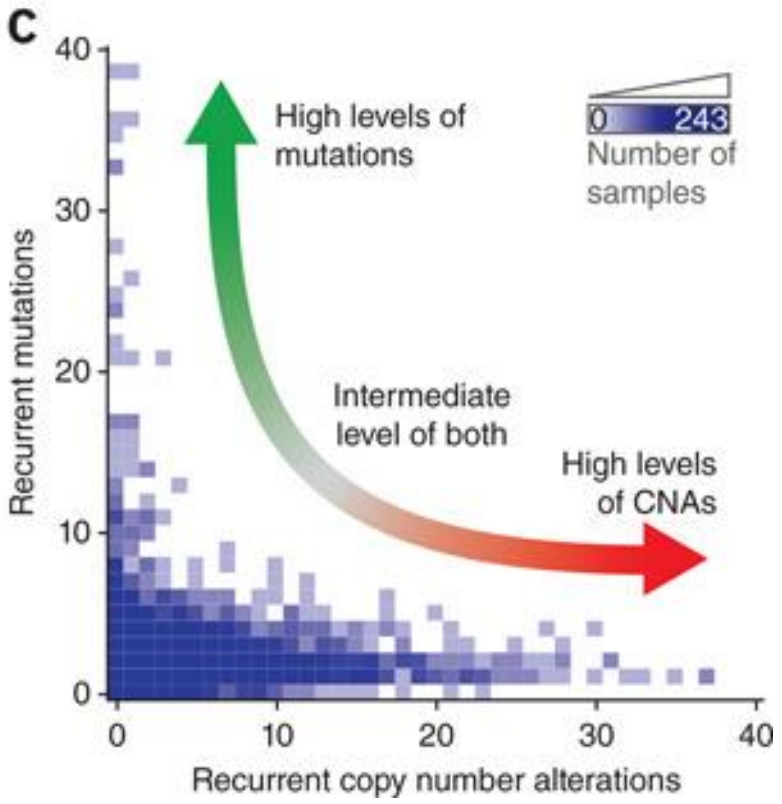
Does mutation rate correlate with aneuploidy?



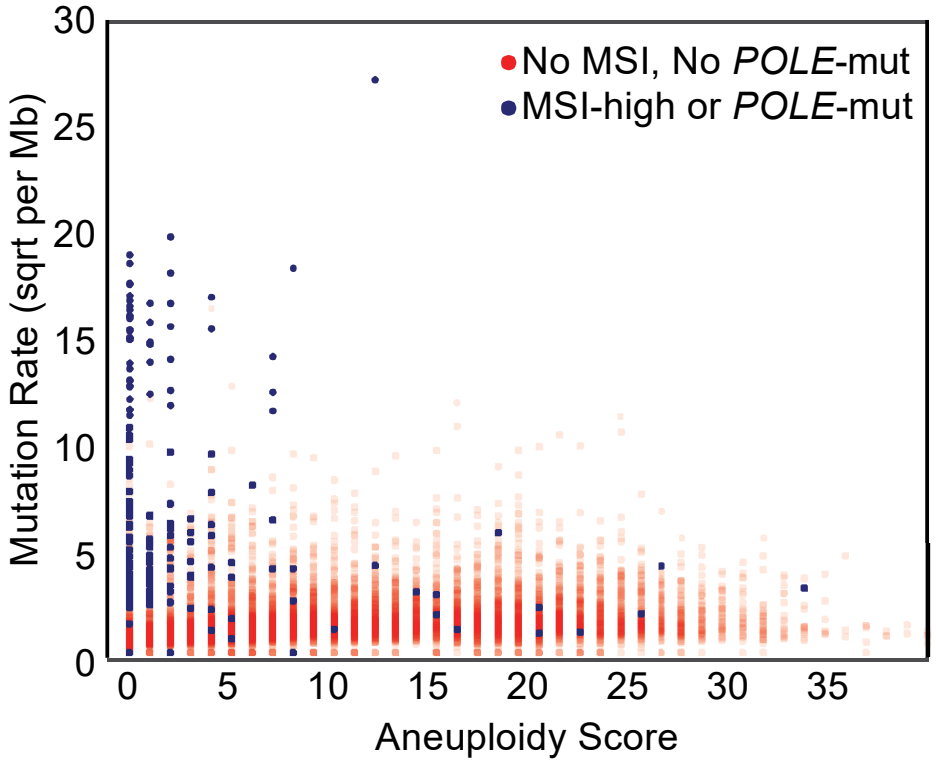


Ciriello et al., 2013

Tumors with Microsatellite Instability have Lower Aneuploidy

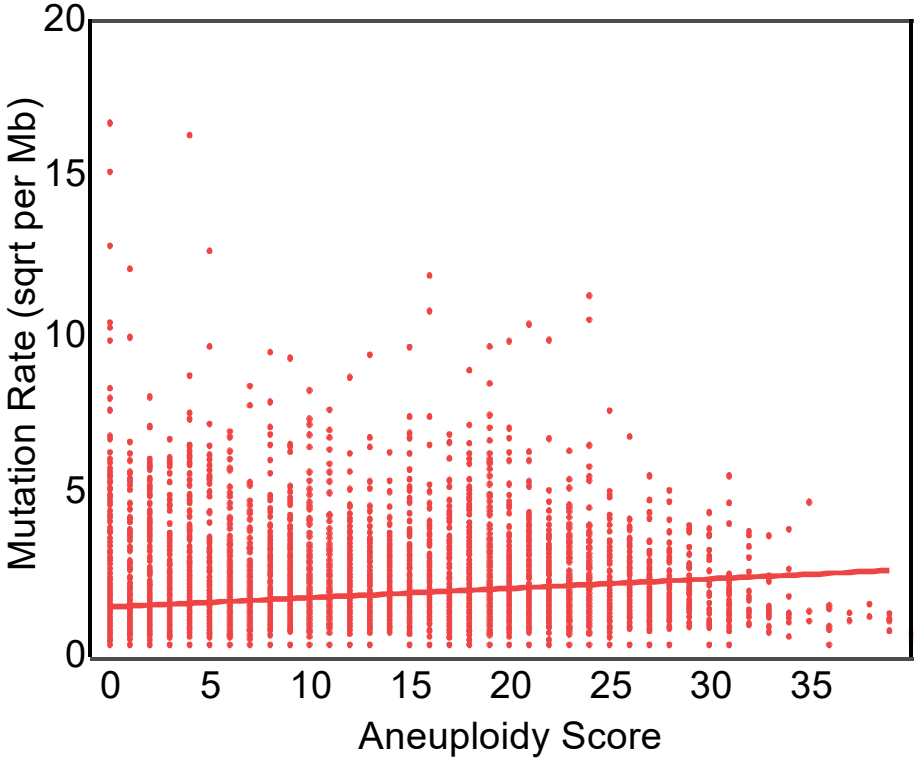


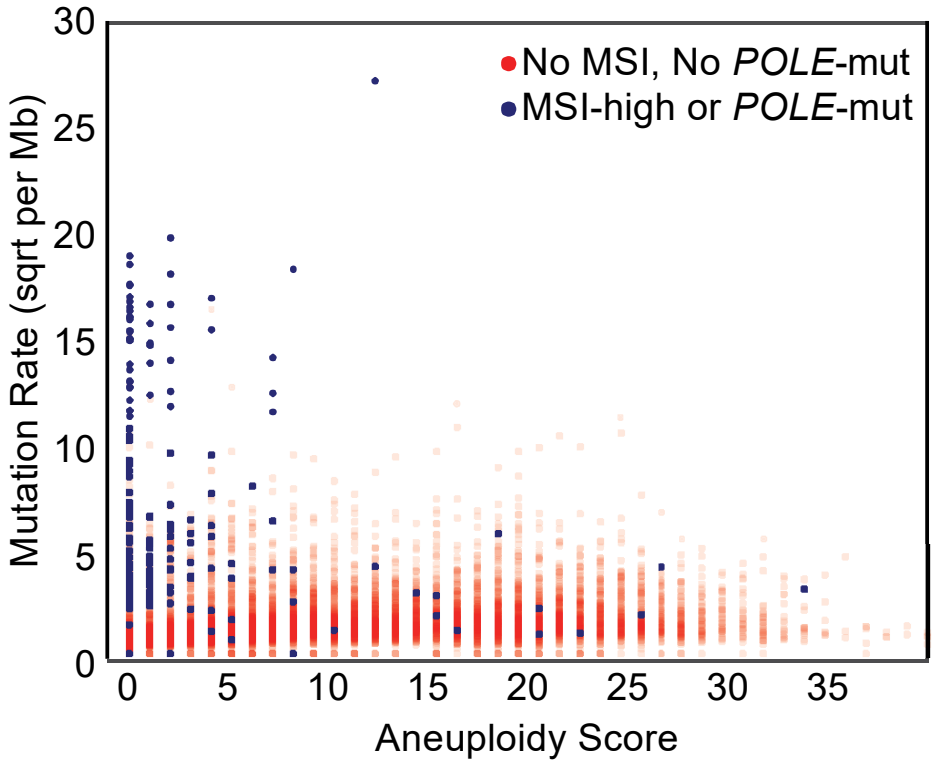
Ciriello et al., 2013



Taylor et al., 2018 *Cancer Cell*

Aneuploidy Positively Correlates with Mutation Rate





Taylor et al., 2018 *Cancer Cell*

Mutation rate and aneuploidy load differ among tumor types

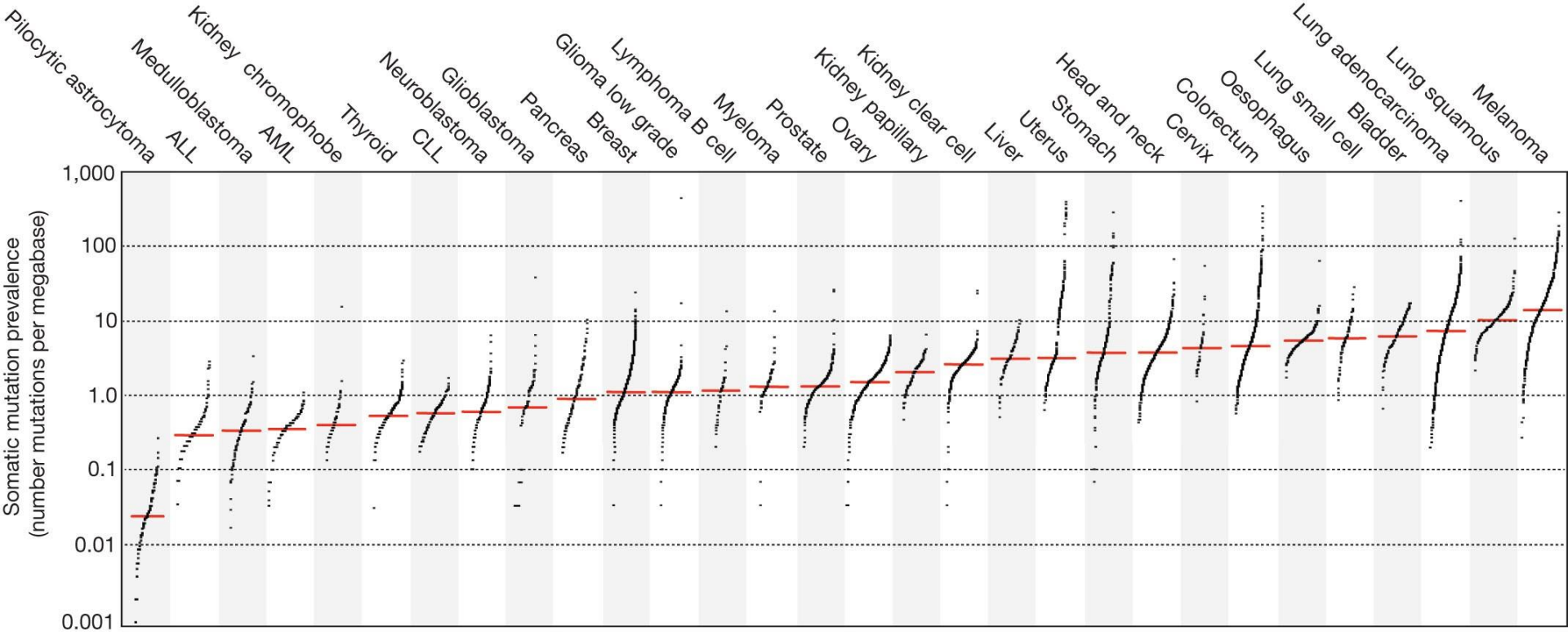
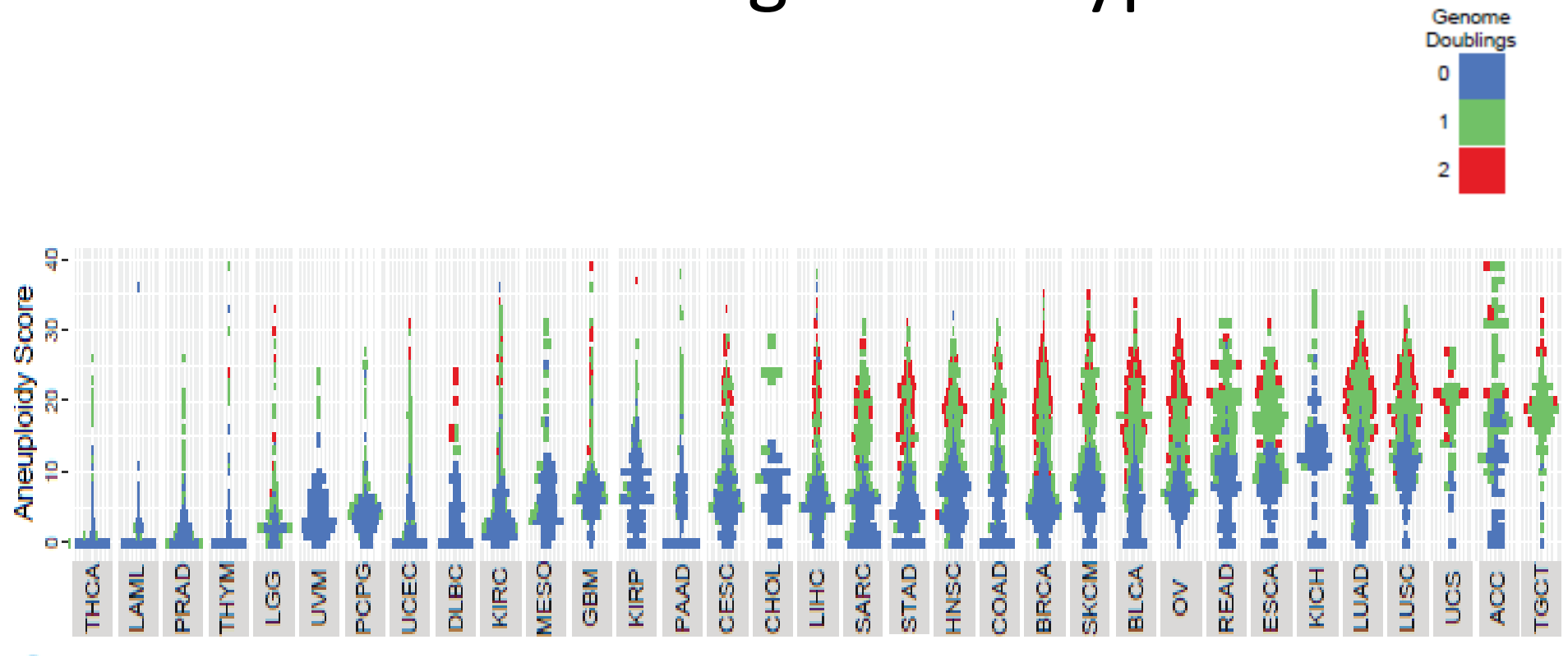


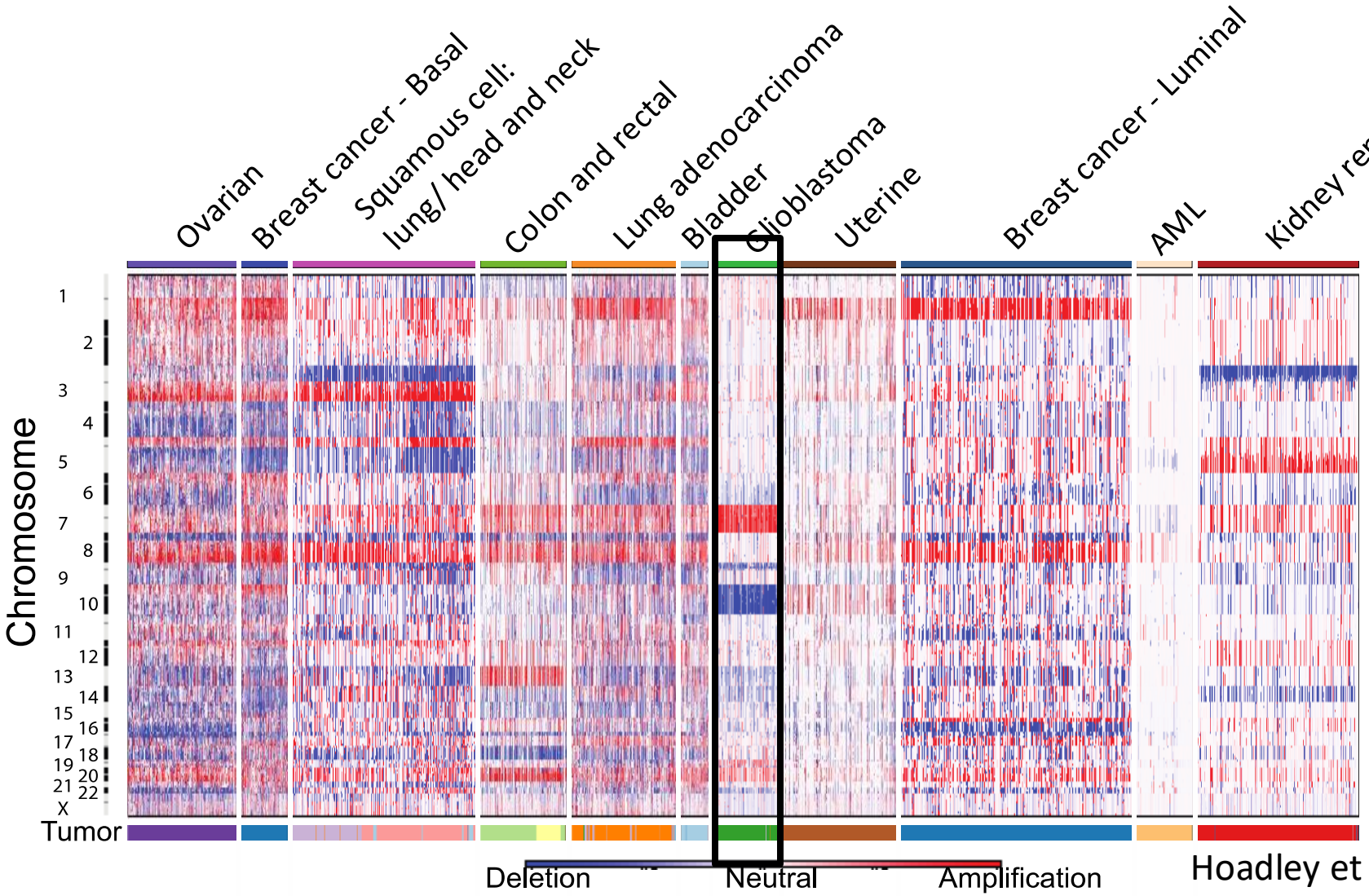
Figure 12.42

Mutation rate and aneuploidy load differ among tumor types



Taylor et al., 2018 *Cancer Cell*

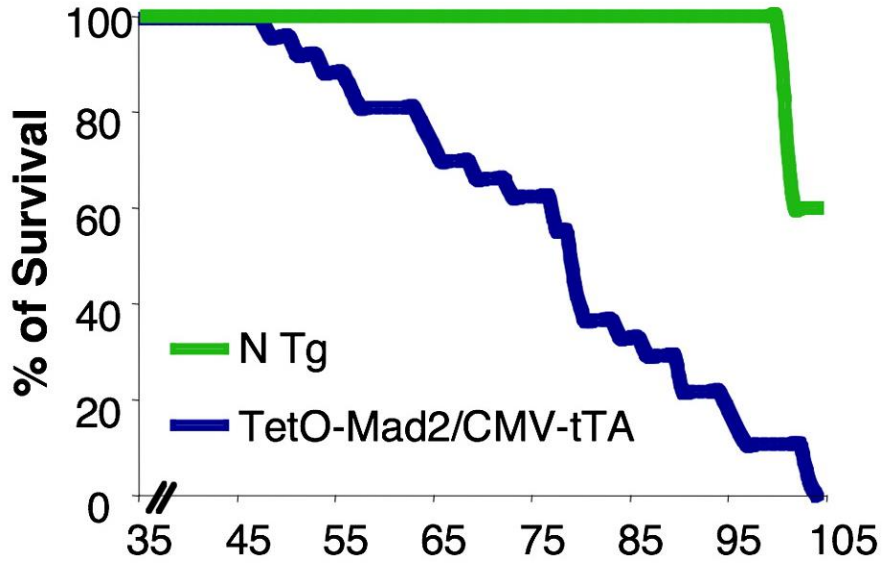
Copy number patterns (and aneuploidy) in cancer show tumor type specificity



Hoadley et al., 2014

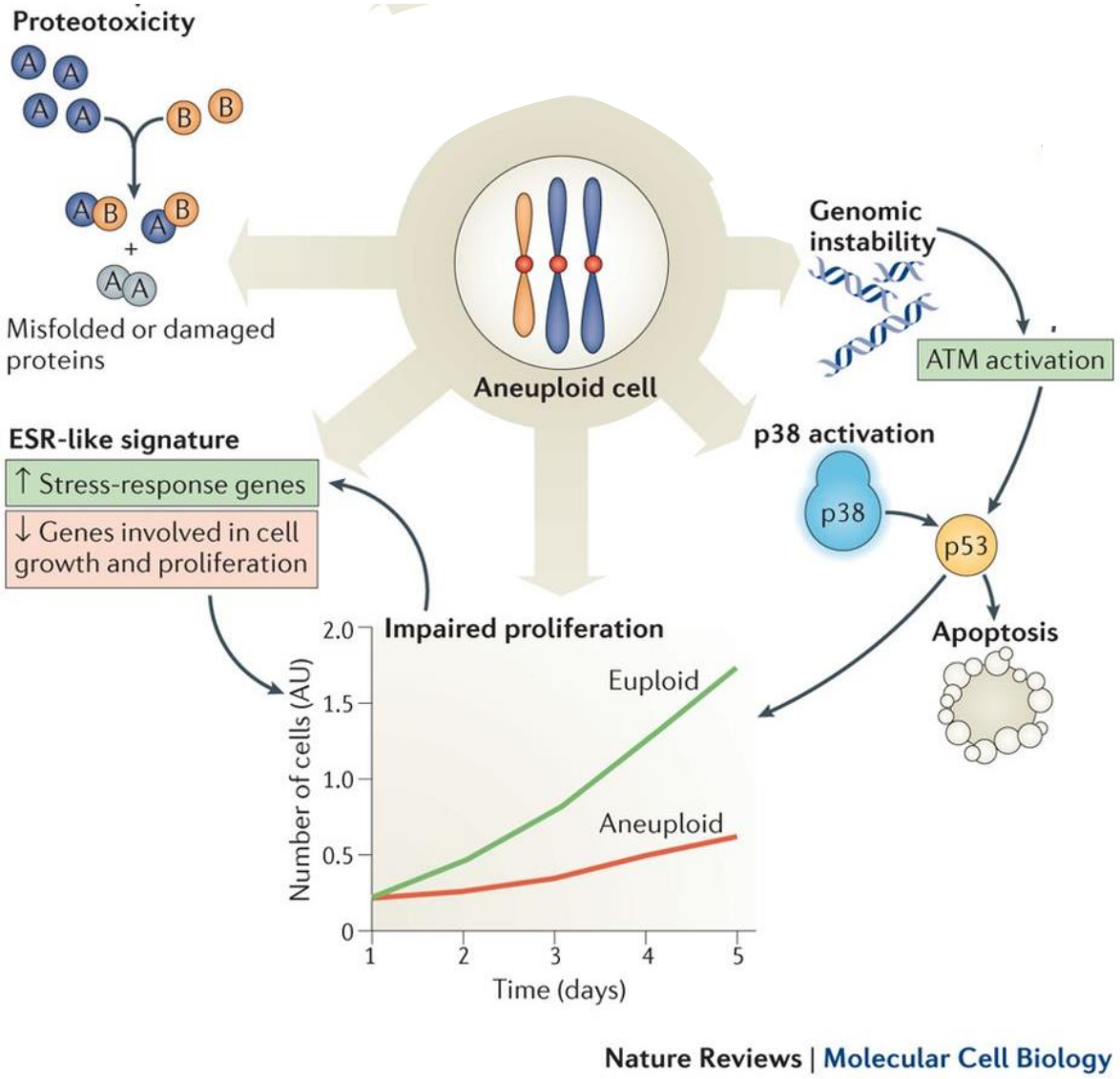
Is aneuploidy an “oncogene”?

Mouse models with mutations in genes required for the spindle assembly checkpoint (like Mad2) have increased tumorigenesis



Sotillo et al., 2007

Individual aneuploidies show decreased cellular fitness



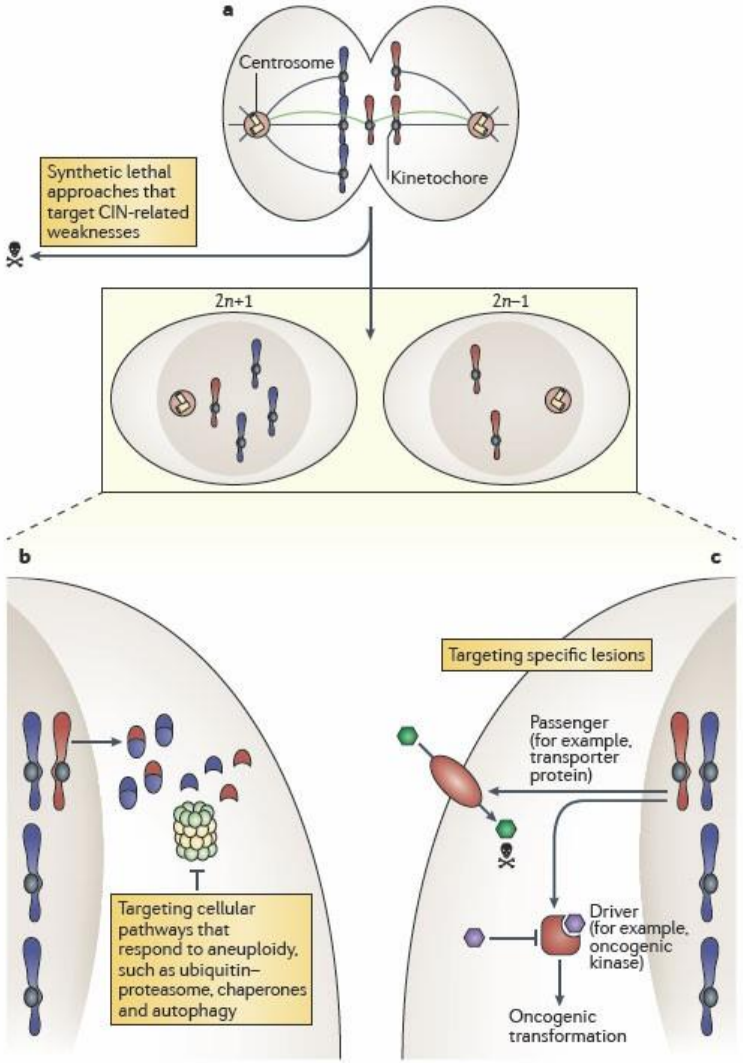
Adapted from Santaguida and Amon, 2015

Outline for today

1. What is genomic instability and chromosome instability? What causes them to occur?
2. What are some tools the cell uses to prevent genomic and chromosome instability (CIN)?
3. What are the consequences of CIN in cancer?
4. **How can we harness this therapeutically?**

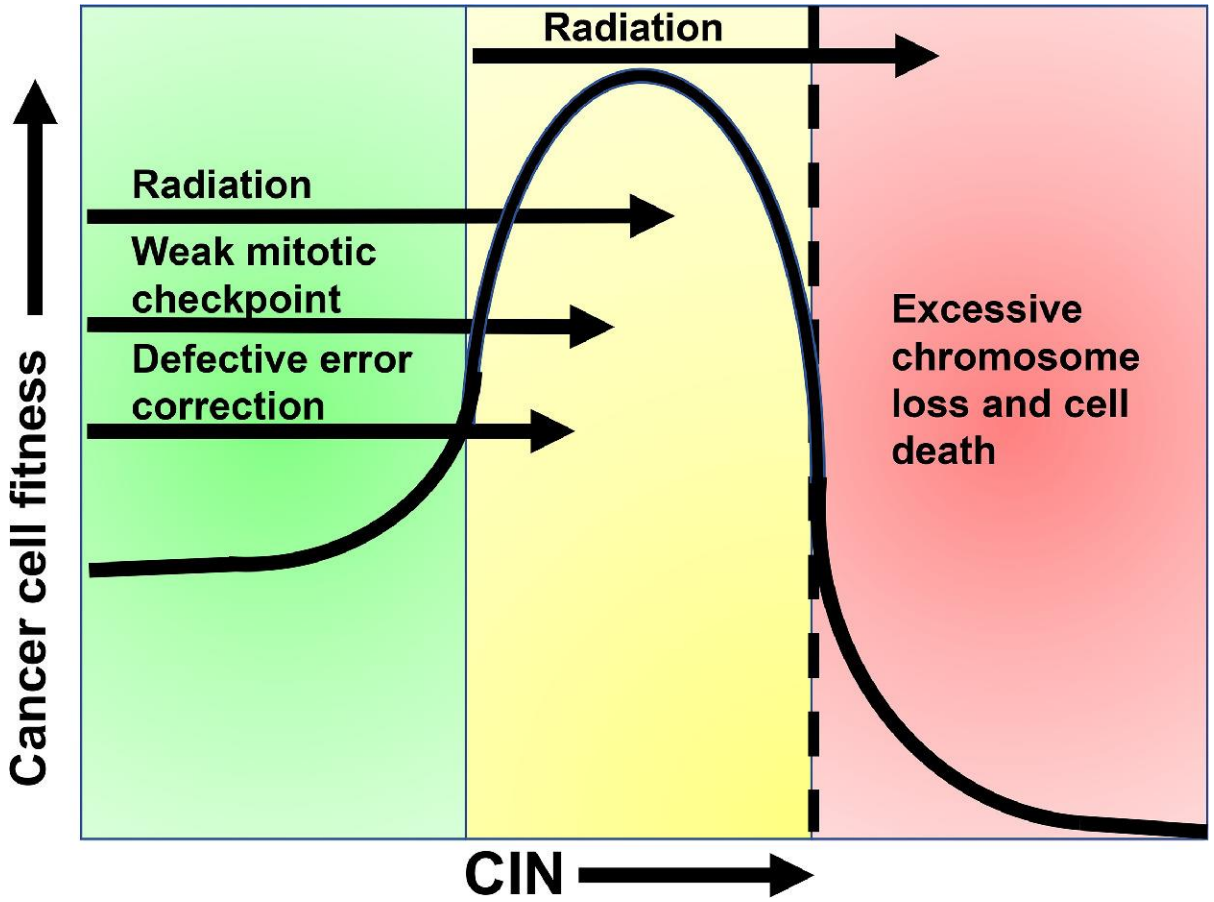
Potential therapeutic approaches

- Synthetic lethal approaches that target CIN-related weaknesses (*KIF18A, radiation)*
- Targeting cellular pathways that respond to aneuploidy (*proteasome, immune*)
- Targeting specific lesions (*paralogs, miRNAs*)



Gordon et al., 2012

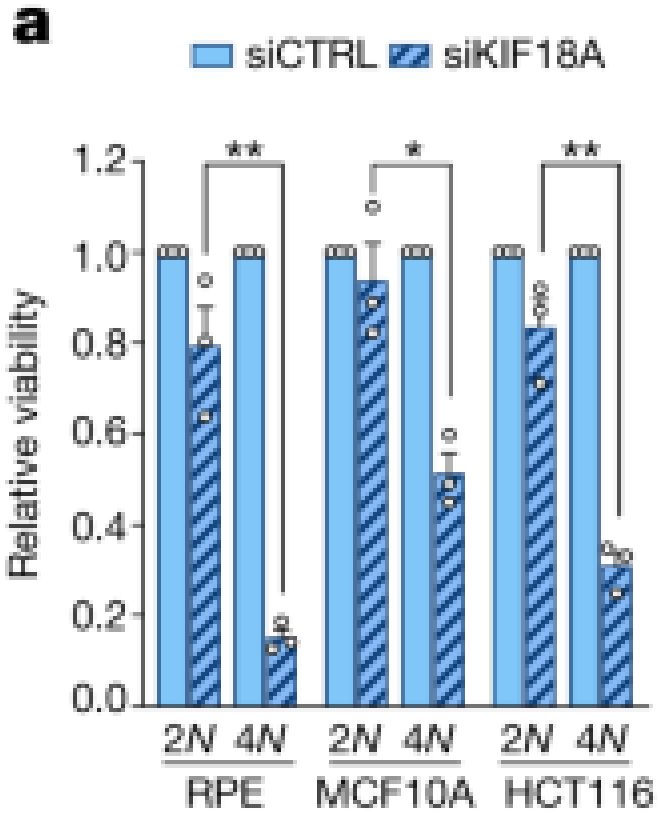
Are aneuploid cells are more sensitive to radiation?



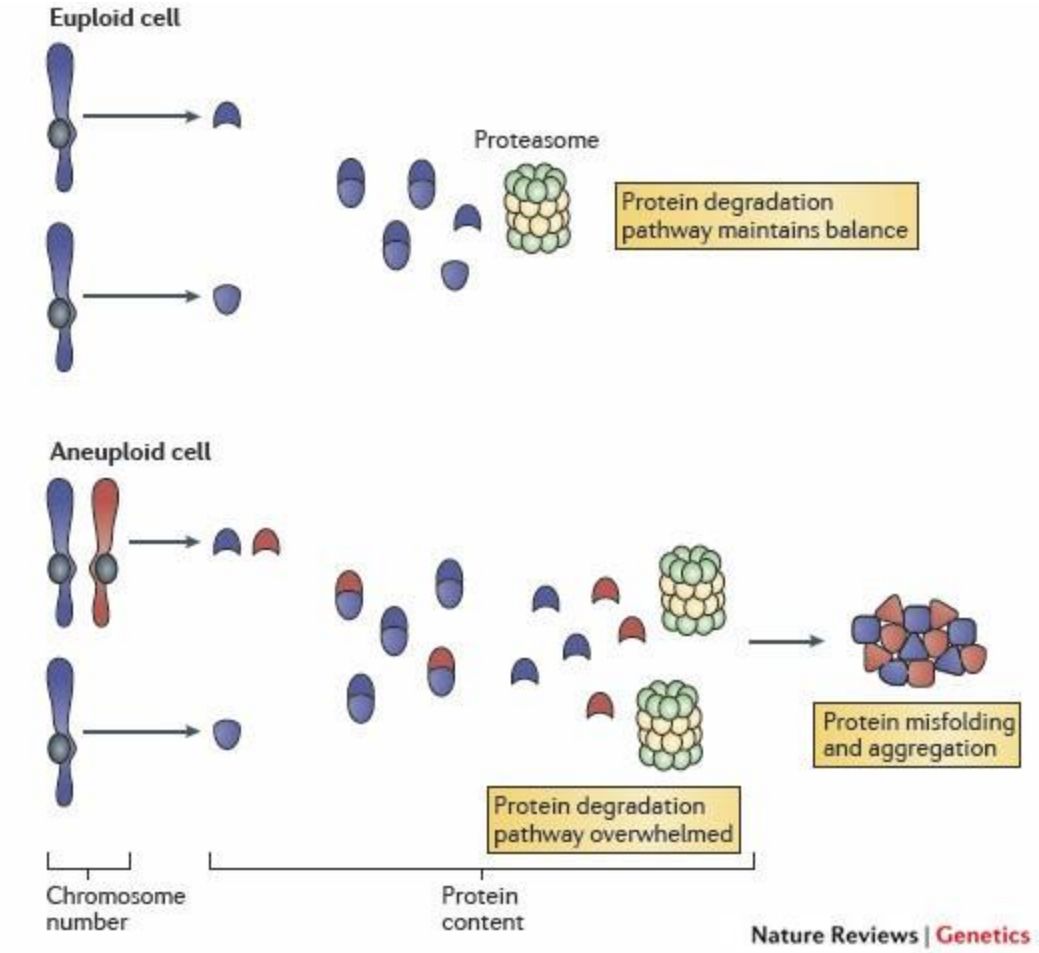
Cosper et al., 2022

Tetraploid cells are more sensitive to inhibition of kinesin *KIF18A*

- Cells with whole genome doubling are more dependent on *KIF18A* knockdown (Quinton et al., and others)
- KIF18 inhibitors are in development by multiple companies

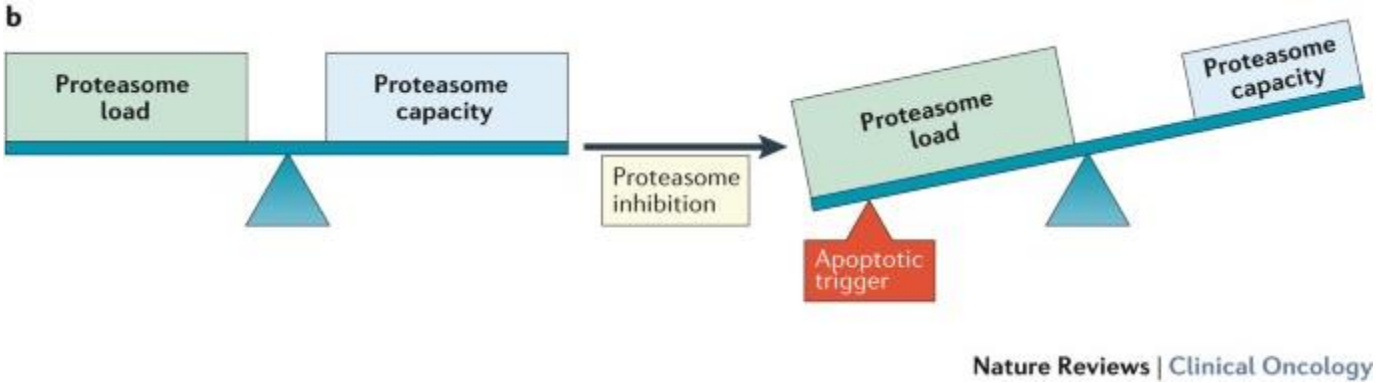


Proteasome pathway is overwhelmed in aneuploid cells



Gordon et al., 2012

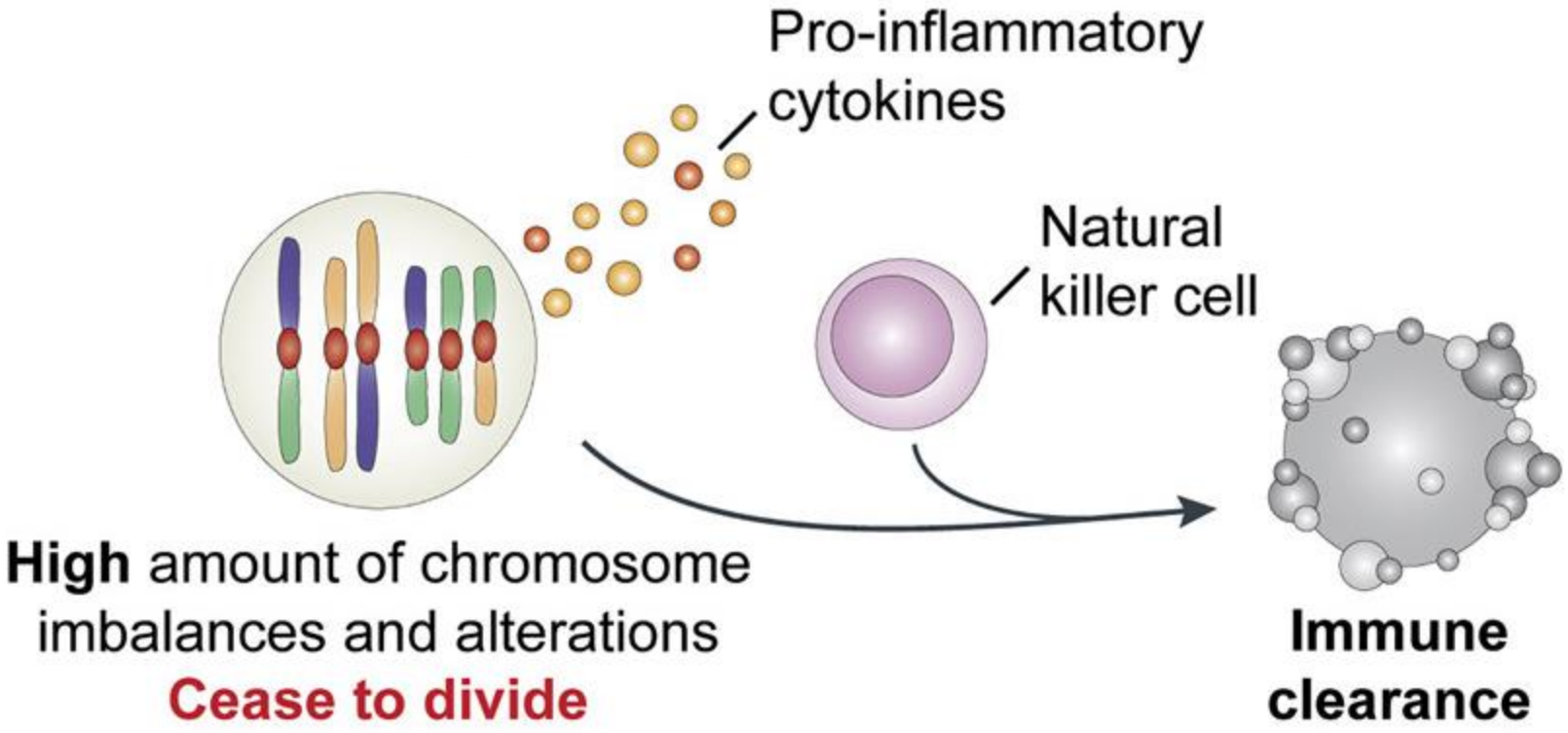
Aneuploid cells are more sensitive to proteasome inhibition



Manasanch & Orlowski, 2017

- Works in the lab, but has not translated clinically
- Proteasome inhibitors are currently effective in multiple myeloma

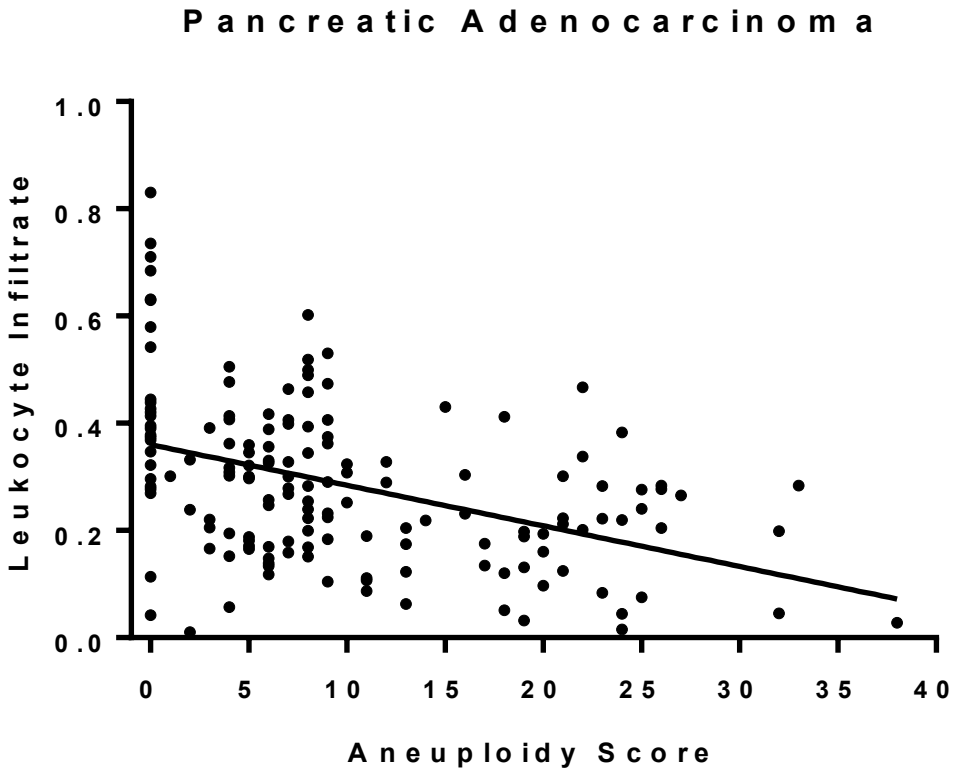
Aneuploidy can induce pro-inflammatory cytokines….



Adapted from Santaguida et al., 2017

…but in cancer aneuploidy anticorrelates with immune infiltrate

- **Leukocyte infiltrate**: estimated using methylation data (Thorsson et al., 2018)
- Negative correlation between aneuploidy and leukocyte fraction



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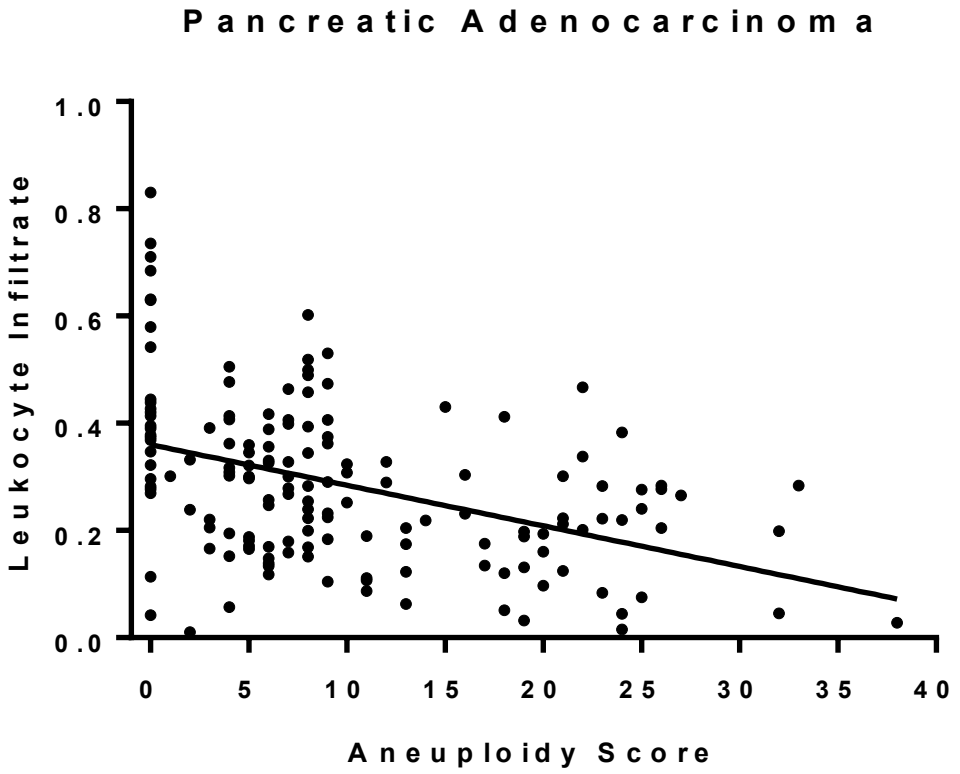


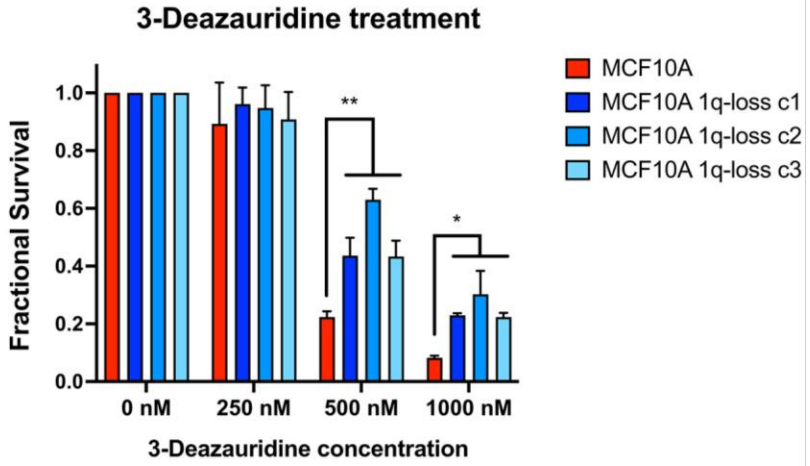
Therapeutic targets based on individual aneuploidy events

RESEARCH ARTICLE | CANCER GENETICS

Oncogene-like addiction to aneuploidy in human cancers

VISHRUTH GIRISH, ASAD A. LAKHANI, SARAH L. THOMPSON, CHRISTINE M. SCADUTO, LEANNE M. BROWN, RYAN A. HAGENSON, ERIN L. SAUSVILLE, BRIANNA E. MENDELSON, PRANAV K. KANDIKUPPA, DEVON A. LUKOW, MONET LOU YUAN, ERIC C. STEVENS, SOPHIA N. LEE, KLASKE M. SCHUKKEN, SARON M. AKALU, ANAND VASUDEVAN, CHARLES ZOU, BARBORA SALOVSKA, WENXUE LI, JOAN C. SMITH, ALISON M. TAYLOR, ROBERT A. MARTIENSSEN, YANSHENG LIU, RUPING SUN, AND JASON M. SHELTZER fewer Authors Info & Affiliations

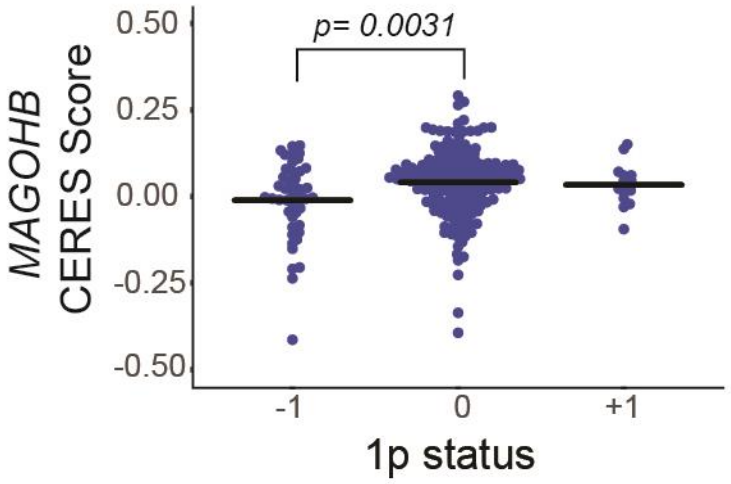
SCIENCE • 6 Jul 2023 • Vol 381, Issue 6660 • DOI: 10.1126/science.adg4521



nature genetics
LETTERS
https://doi.org/10.1038/s41588-018-0155-3

Genome-scale analysis identifies paralog lethality as a vulnerability of chromosome 1p loss in cancer

Srinivas R. Viswanathan[1,2,3], Marina F. Nogueira[1,2,12], Colin G. Buss[4,5,12], John M. Krill-Burger[2], Mathias J. Wawer[6], Edyta Malolepsza[2,7], Ashton C. Berger[1,2], Peter S. Choi[1,2,3], Juliann Shih[2], Alison M. Taylor[1,2,3], Benjamin Tanenbaum[2], Chandra Sekhar Pedamallu[1], Andrew D. Cherniack[2], Pablo Tamayo[2,8], Craig A. Strathdee[2], Kasper Lage[2,7], Steven A. Carr[2], Monica Schenone[2], Sangeeta N. Bhatia[2,3,4,5,9,10,11], Francisca Vazquez[2], Aviad Tsherniak[2], William C. Hahn[1,2,3] and Matthew Meyerson[1,2,3]*



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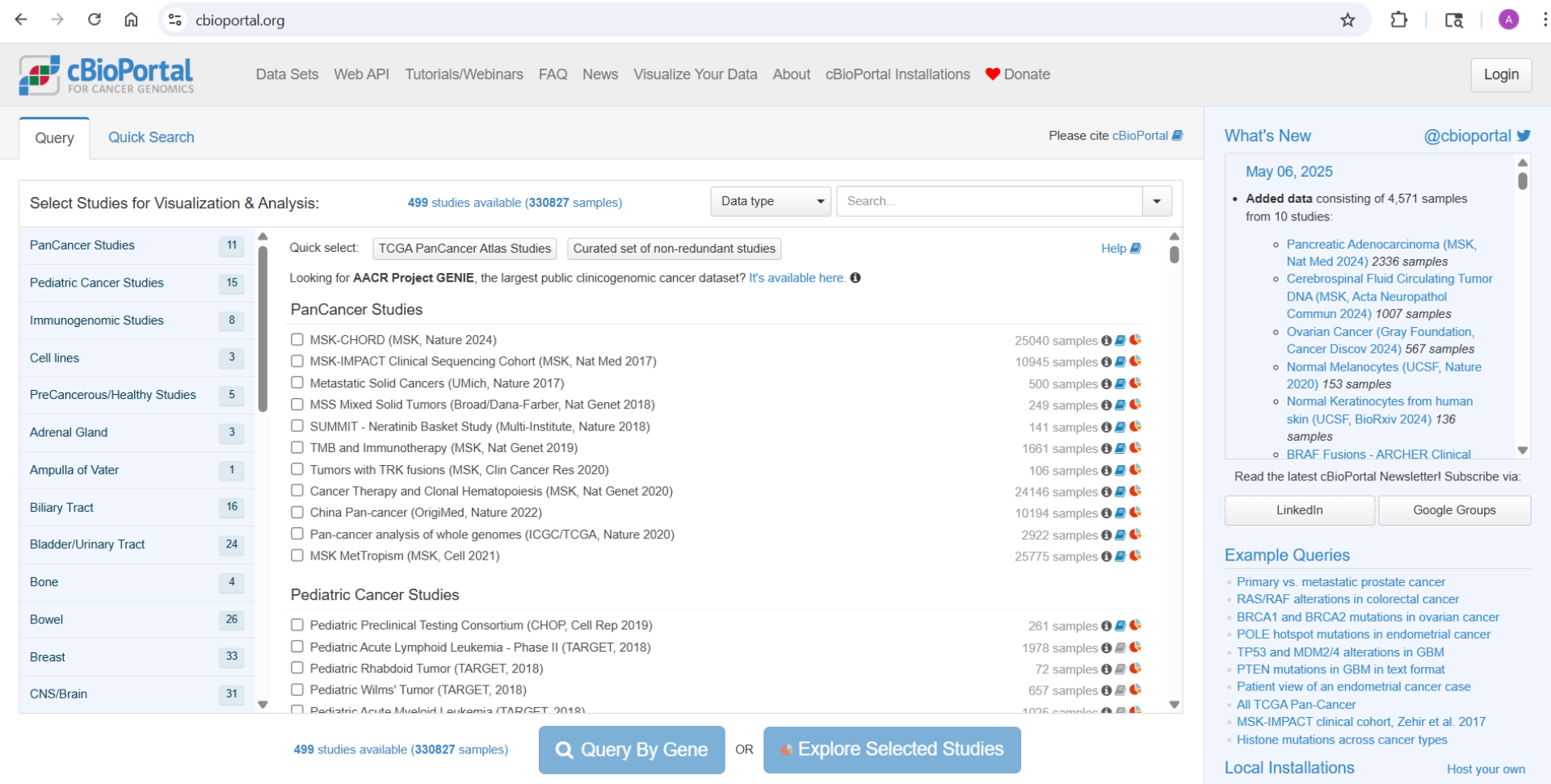
In class cbioportal tutorial



In class cbioportal tutorial

- What cancer type?
- What gene(s) are frequently altered in this cancer type?

Questions we will answer:

- Do alterations in these genes co-occur? Or are they mutually exclusive?
- Does expression correlate with copy number?

Questions?