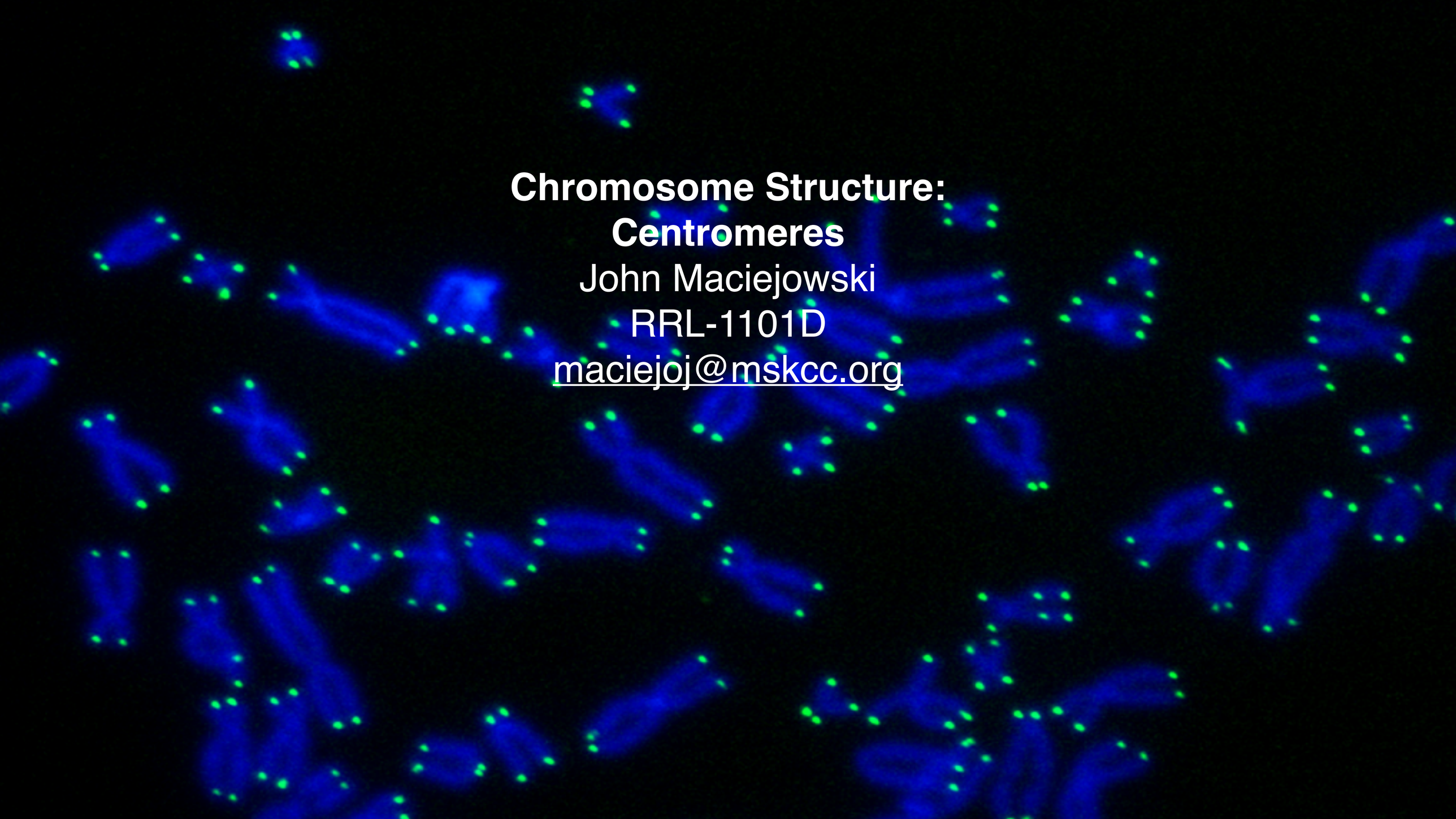

Chromosome Structure: Centromeres

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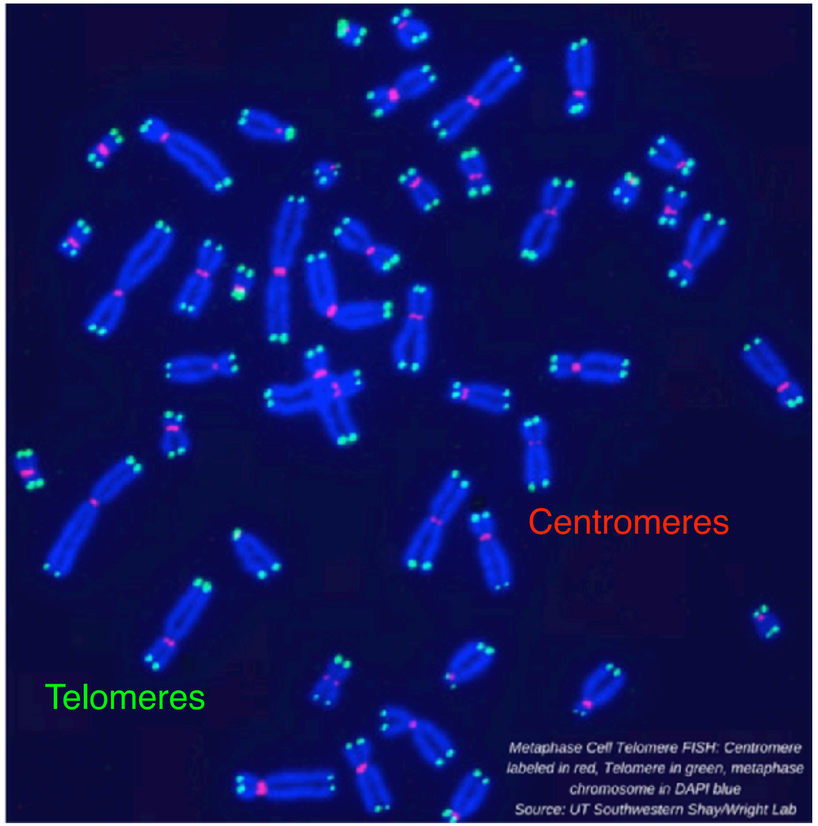
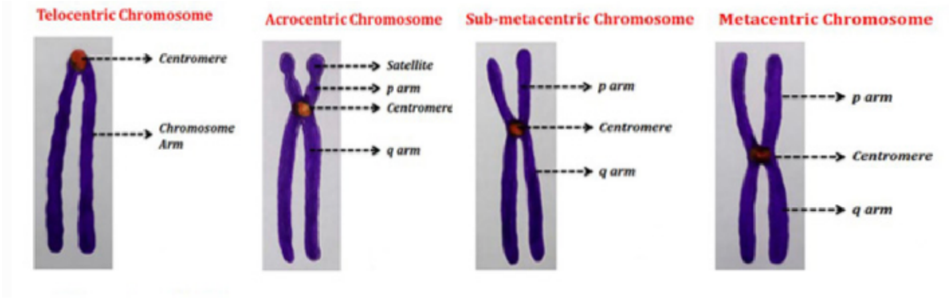
Centromeres are the chromosomal sites of kinetochore assembly





What defines centromere identity?
Epigenetically defined via specialized histone H3 variant named CENP-A

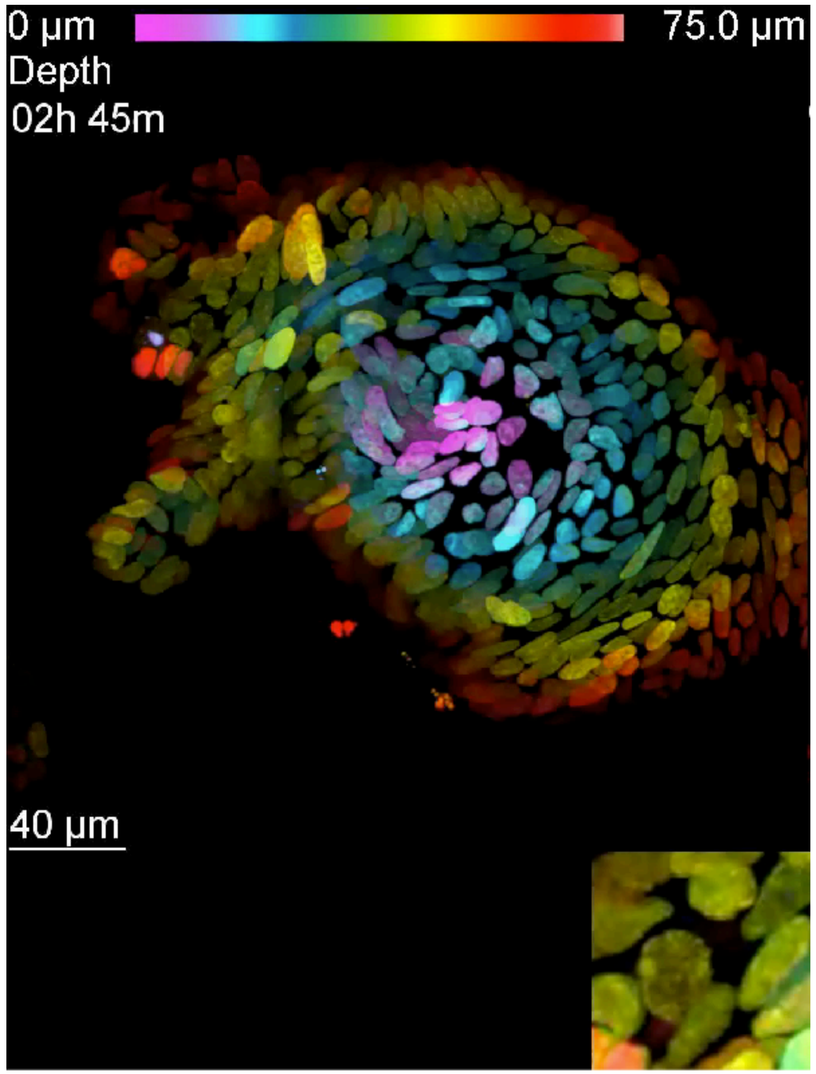
What is the function of the centromere?
Region of a chromosome where the kinetochore is assembled during mitosis

What are the consequences of centromere instability?
Aneuploidy, structural rearrangements, innate immune activation

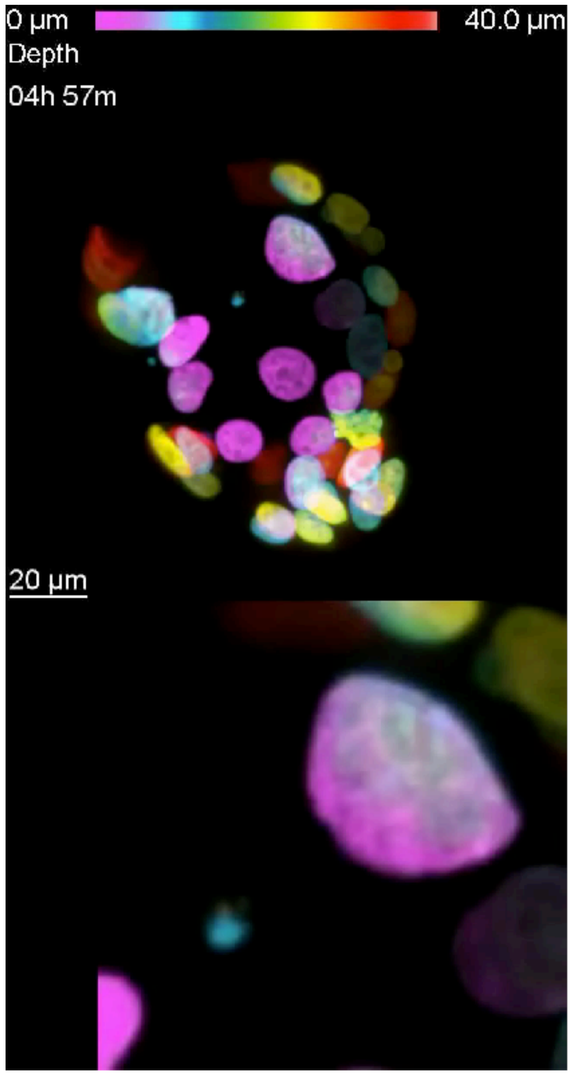
Time-lapse movie of mitosis in African blood lily

Modern approaches to imaging mitosis

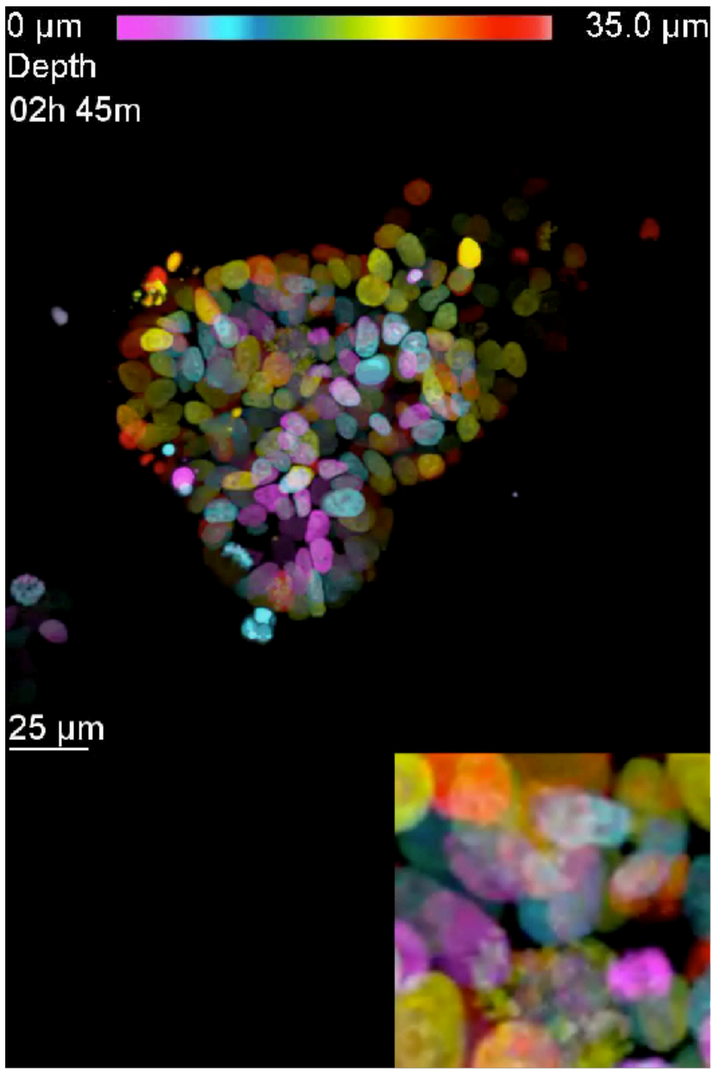
Normal Division



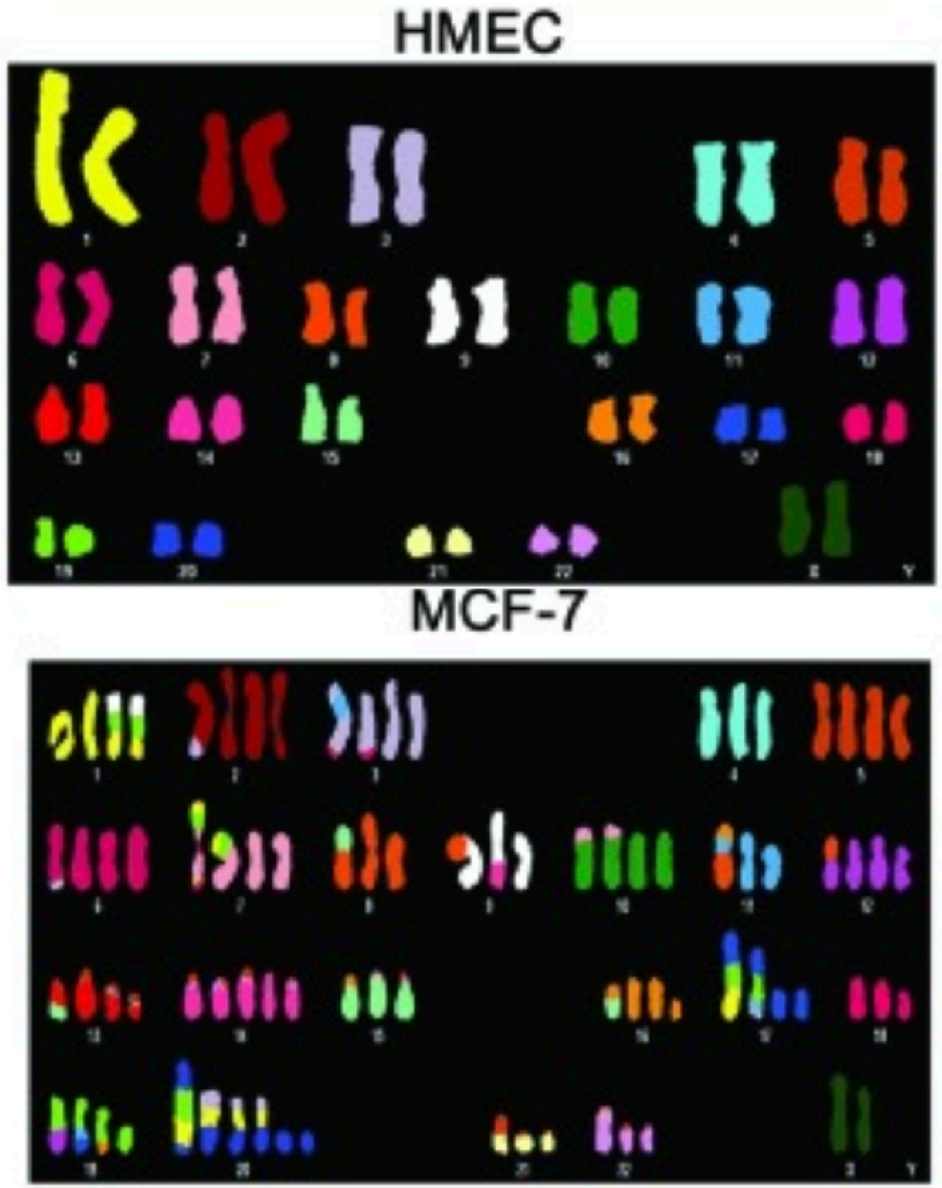
Lagging Chromosome



Multipolar Division



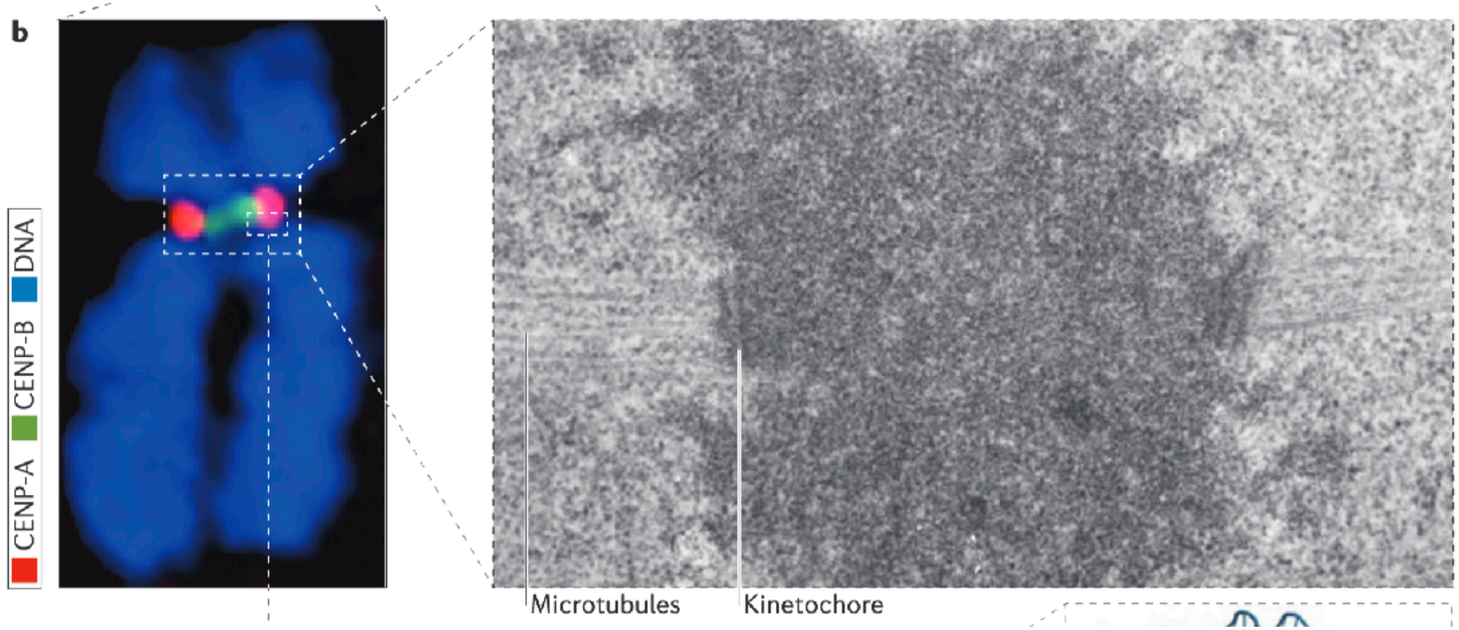
Aneuploidy is a common feature of cancer



Aneuploidy

- abnormal number of chromosomes
- common cause of genetic disorders
- >70% of human solid tumors are aneuploid
- caused by errors in cell division

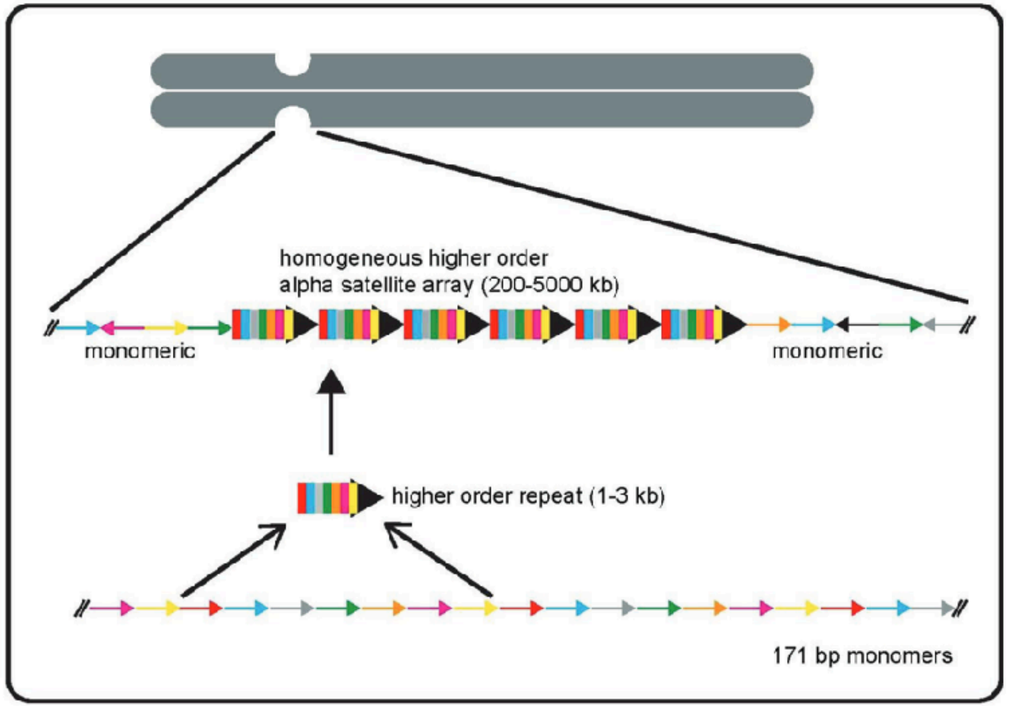
Centromere function was apparent early on



Cyril Darlington (1936): “[the centromere must] be considered in terms of function rather than form, since the function is evident and the form elusive”

Centromeric DNA

Proteins associated with centromeres are conserved, but DNA sequences are dissimilar - not only among organisms, but often within the same organism



α-satellite DNA composed of 171 bp monomers

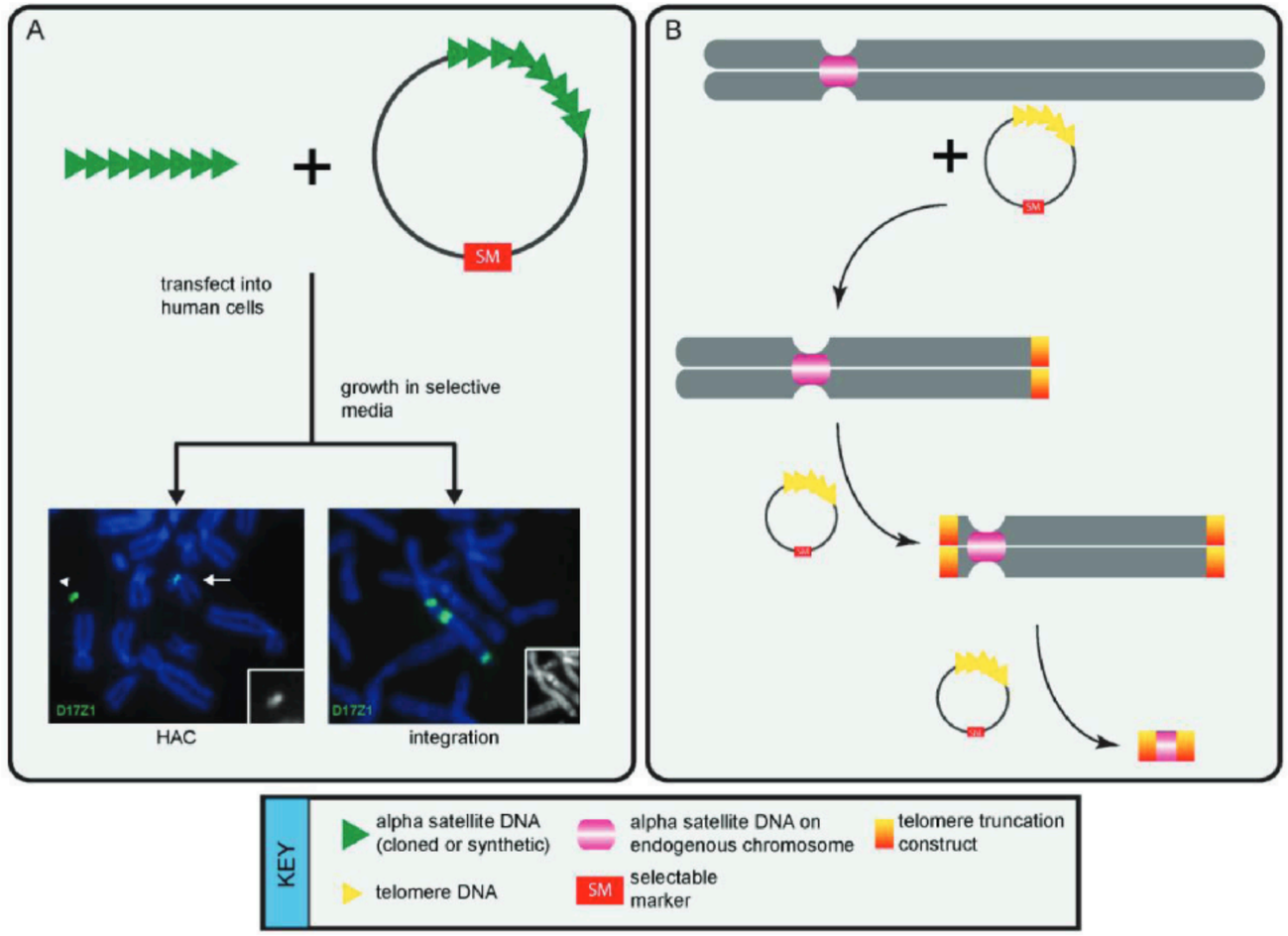
Monomers differ by up to 40%

Repeats can span megabases

α-satellite DNA makes up 3-5% of human genome

Is α-satellite DNA important for human centromere function?

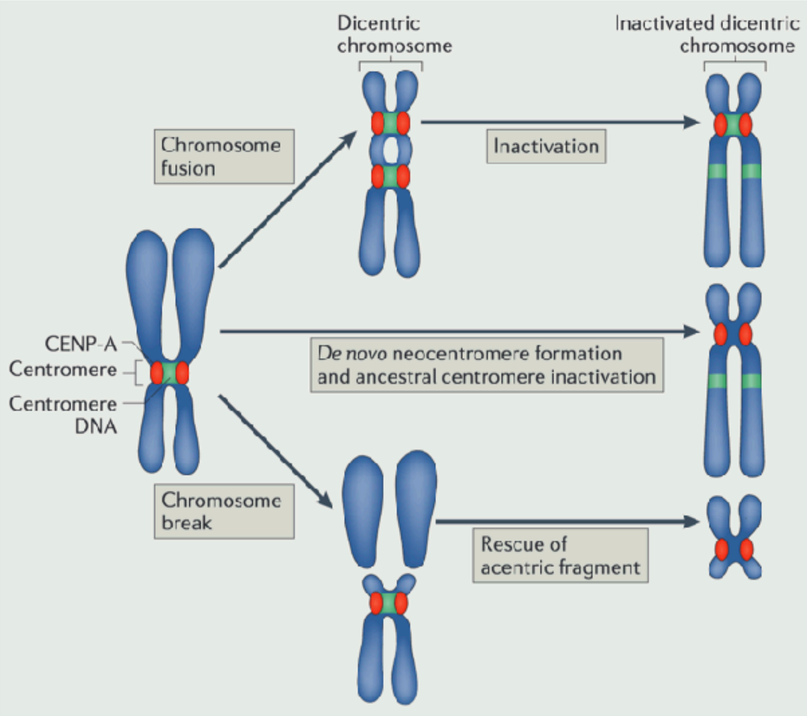
Minichromosomes define α-satellite as the functional human centromere



HOR α-satellite DNA is a preferred substrate for *de novo* centromere assembly

α-satellite DNA is not necessary or sufficient for centromere formation

Evidence for the epigenetic nature of the centromere

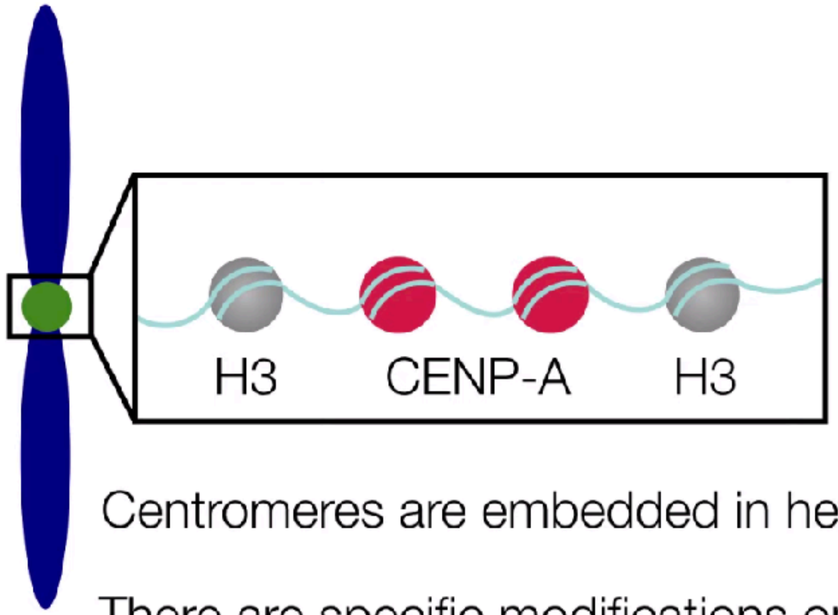


Centromeres can be inactivated without changes to the underlying DNA sequence.

Neocentromeres can also form in the absence of α-satellite repeats.

Neocentromeres recruit all known centromere associated proteins, including CENP-A

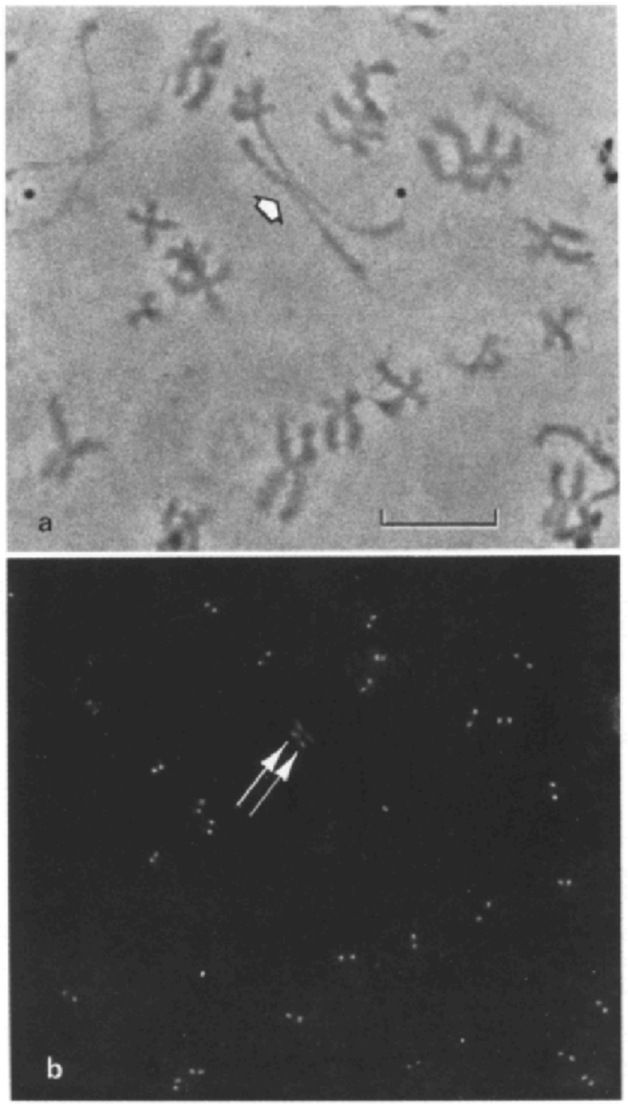
Centromeres are specified epigenetically



Centromeres are embedded in heterochromatin

There are specific modifications on the histones in centromeres

CREST syndrome patients have anti-centromere antibodies



CREST syndrome: scleroderma spectrum disease

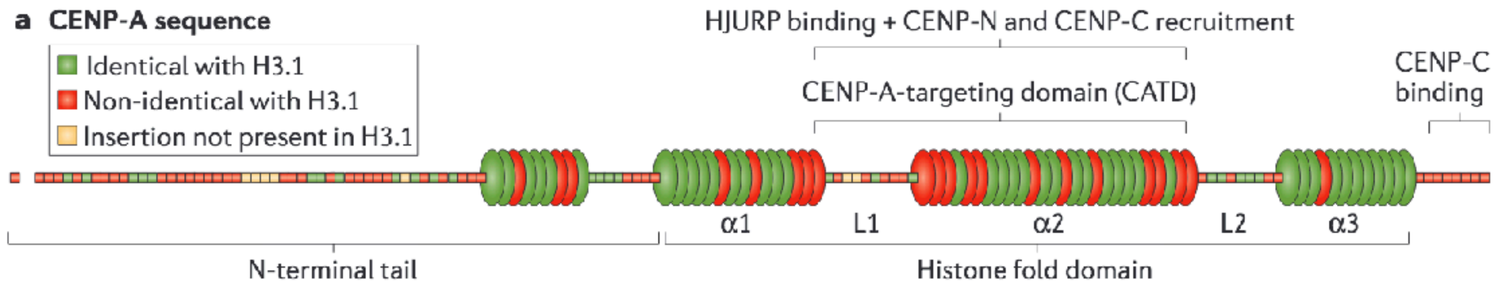
First evidence of centromere-specific proteins

CENP-A, CENP-B, & CENP-C are recognized by sera from CREST patients

CENP-A and CENP-C localize to the inner kinetochore

CENP-B is a α-satellite DNA binding protein

CENP-A is an epigenetic hallmark of centromeres



CENP-A exhibits biochemical similarity to Histone H3

CENP-A is essential for the localization of all known kinetochore components

CENP-A is found at all neocentromeres

CENP-A directly interacts with CENP-C and CENP-N

CENP-A-H4 tetramers are more conformationally rigid that H3-H4 tetramers

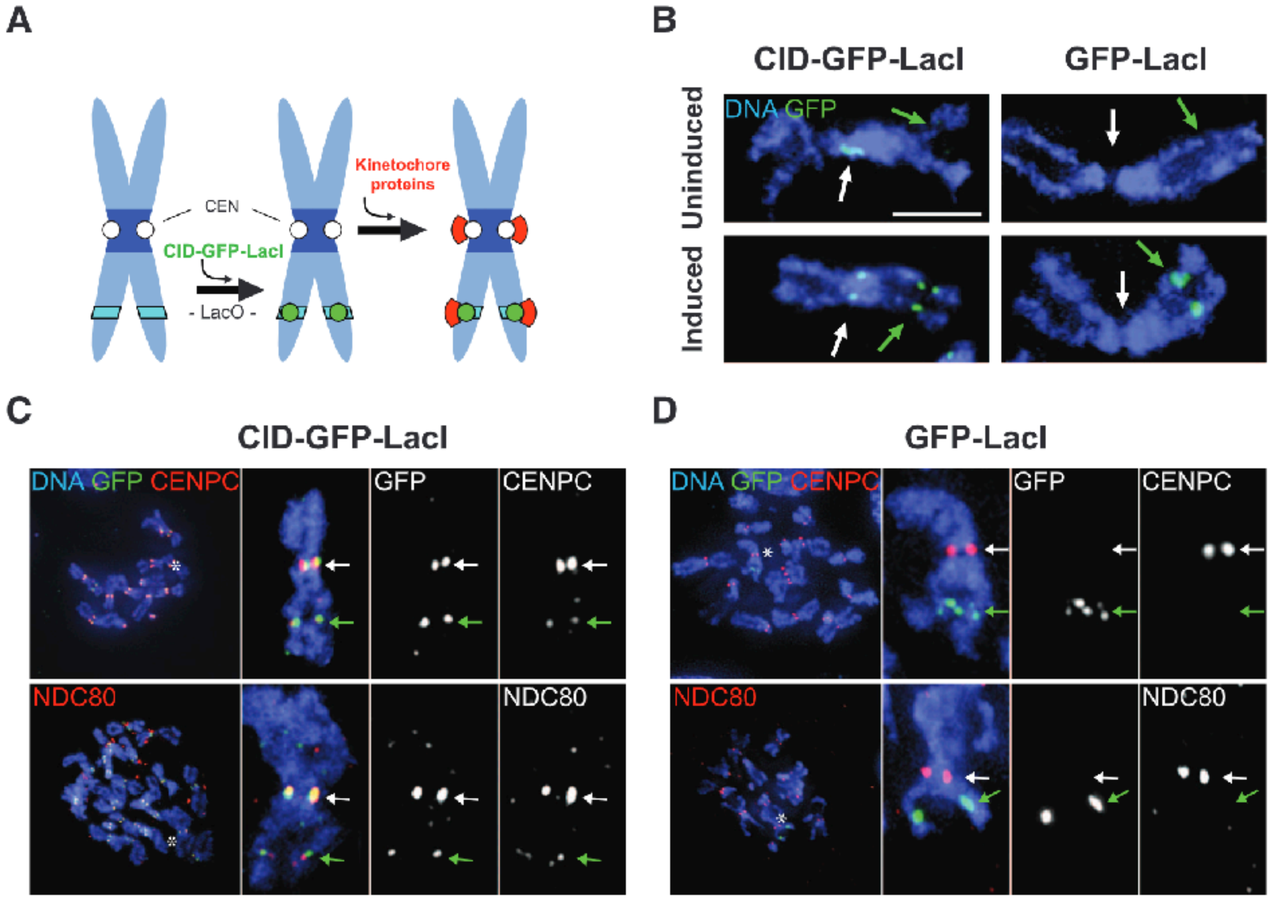
How to determine if CENP-A is sufficient to generate structures capable of directing microtubule attachment & chromosome segregation?

How to determine if CENP-A is sufficient to generate structures capable of directing microtubule attachment & chromosome segregation?

Ectopic CENP-A targeting generates microtubule attachment site

Drosophila CENH3 Is Sufficient for Centromere Formation

María José Mendiburo,[1,2] Jan Padeken,[1,2] Stefanie Fülöp,[3] Aloys Schepers,[3] Patrick Heun[1,4,*]



Ectopic CENP-A targeting generates microtubule attachment site

Drosophila CENH3 Is Sufficient for Centromere Formation

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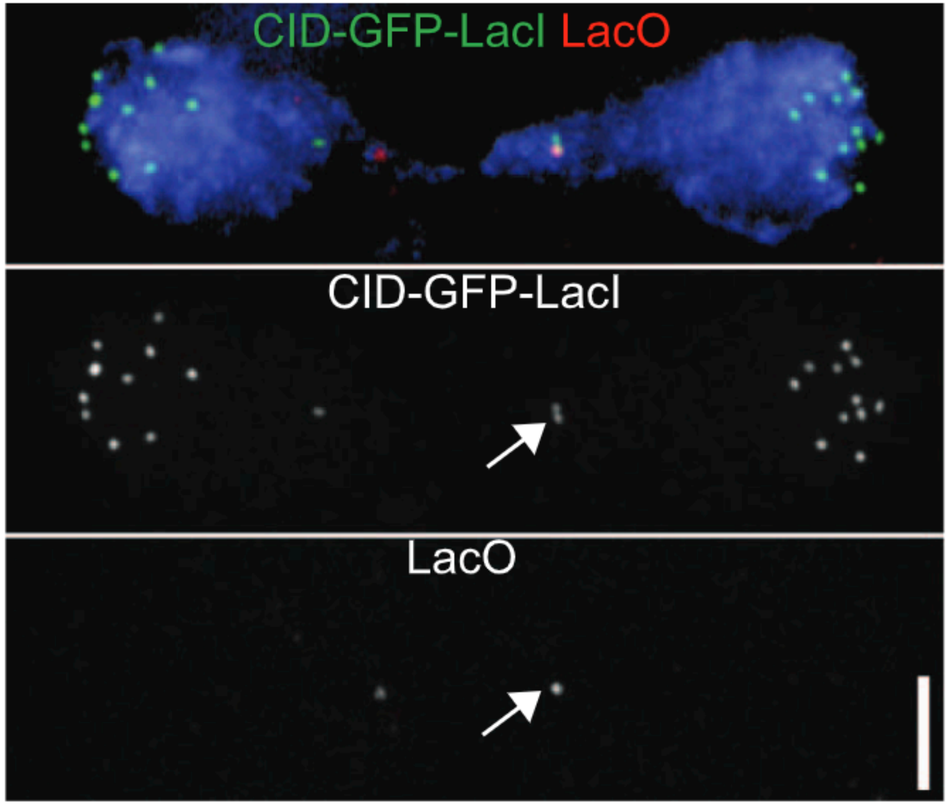
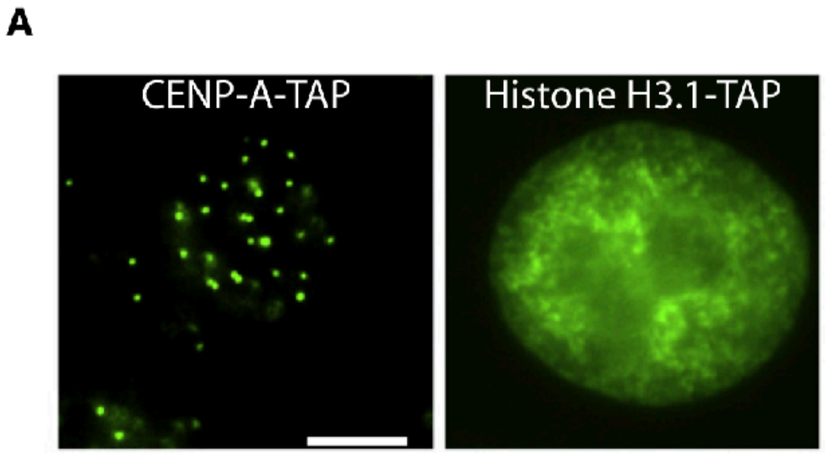
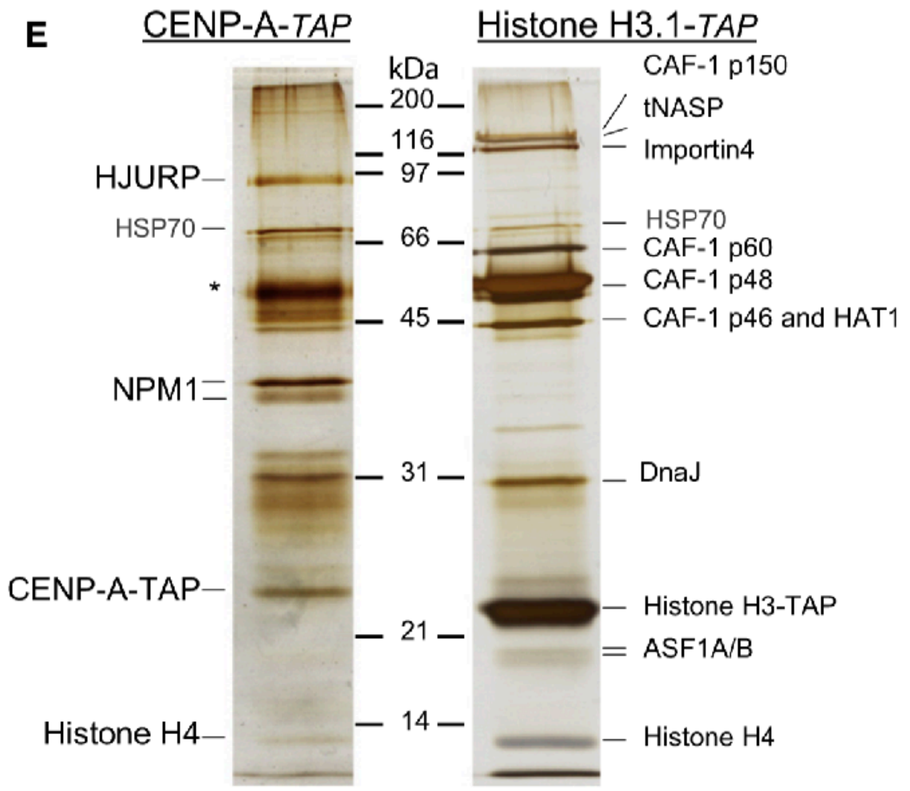
C



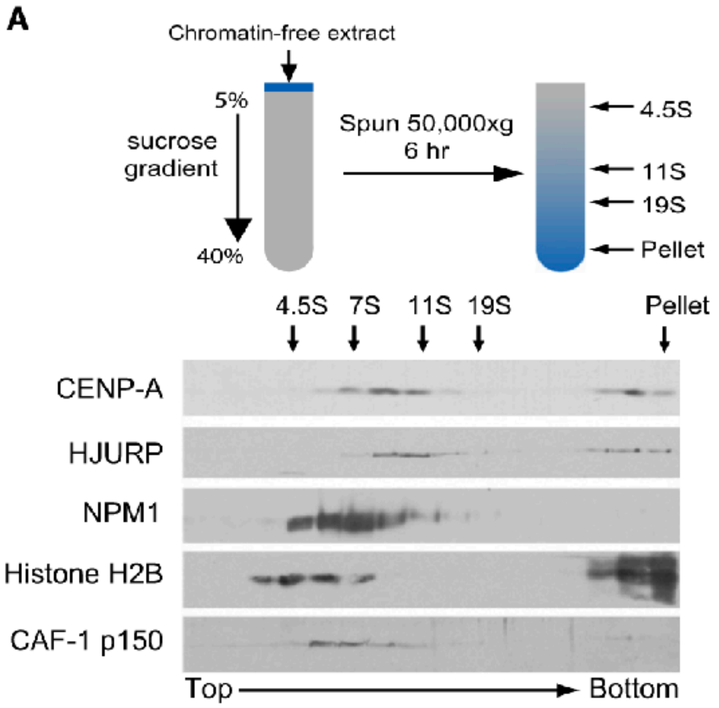
How are centromeres faithfully inherited at a single site
On each chromosome?

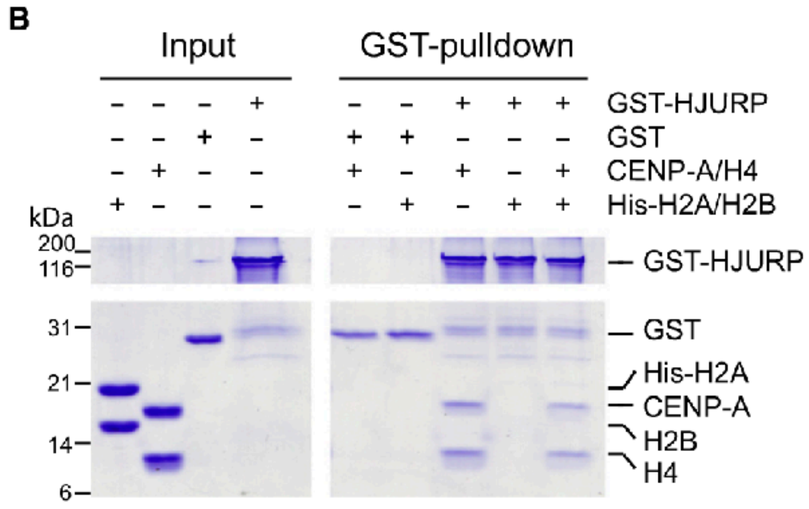
Centromere-specific assembly of CENP-A nucleosomes is mediated by HJURP



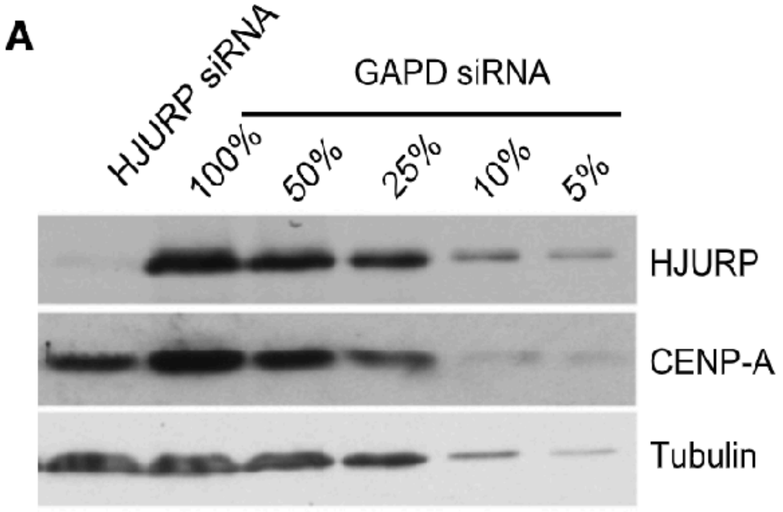


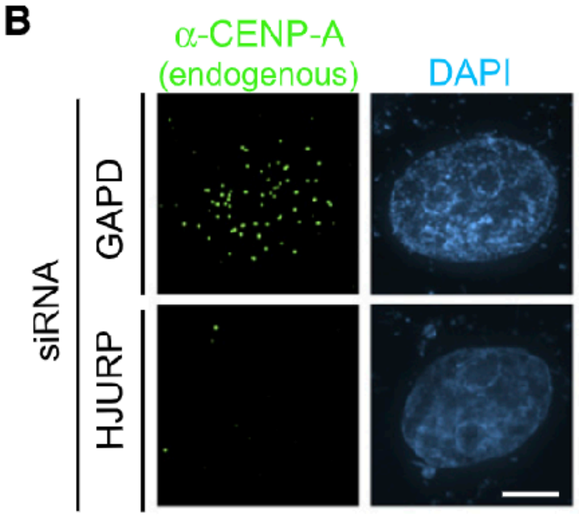
HJURP directly binds CENP-A/Histone H4



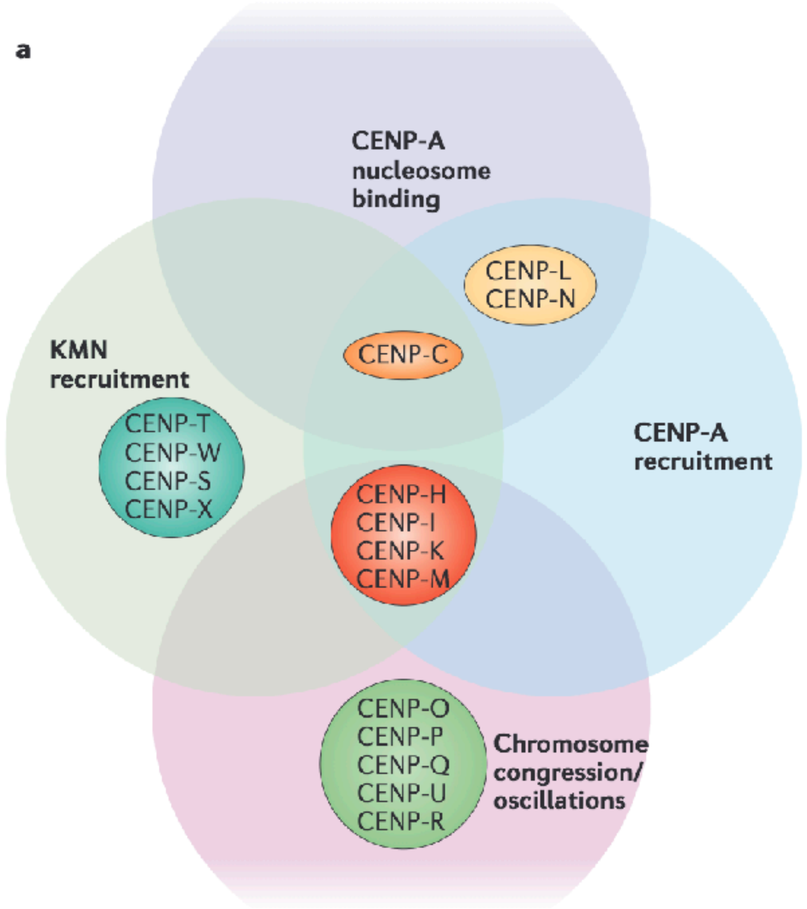


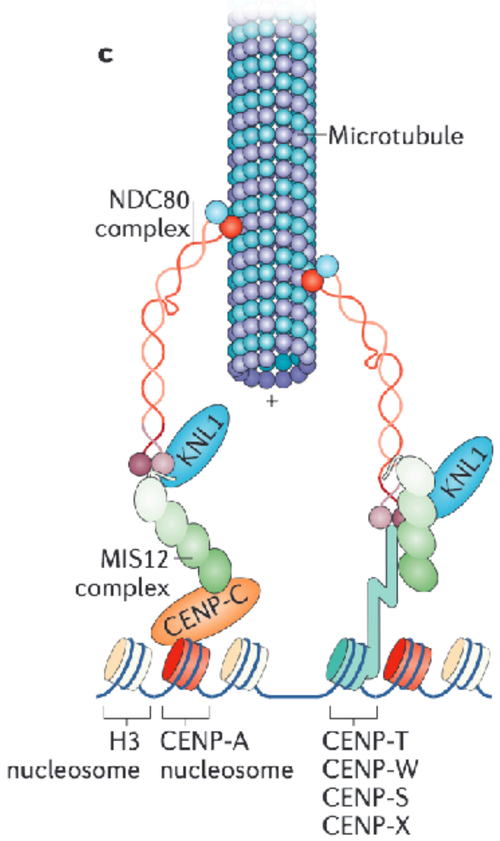
Loss of CENP-A Recruitment in HJURP-depleted cells



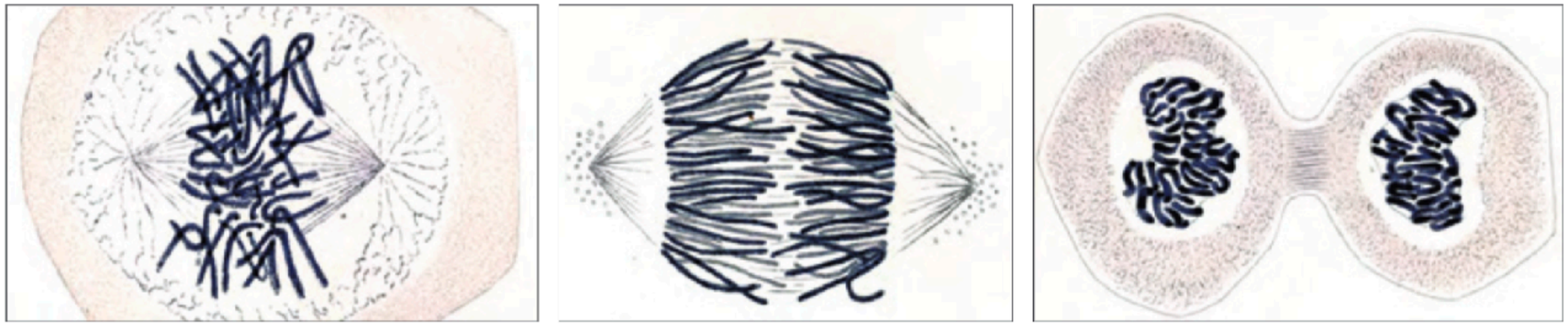


CENP-A builds the kinetochore by recruiting the CCAN complex

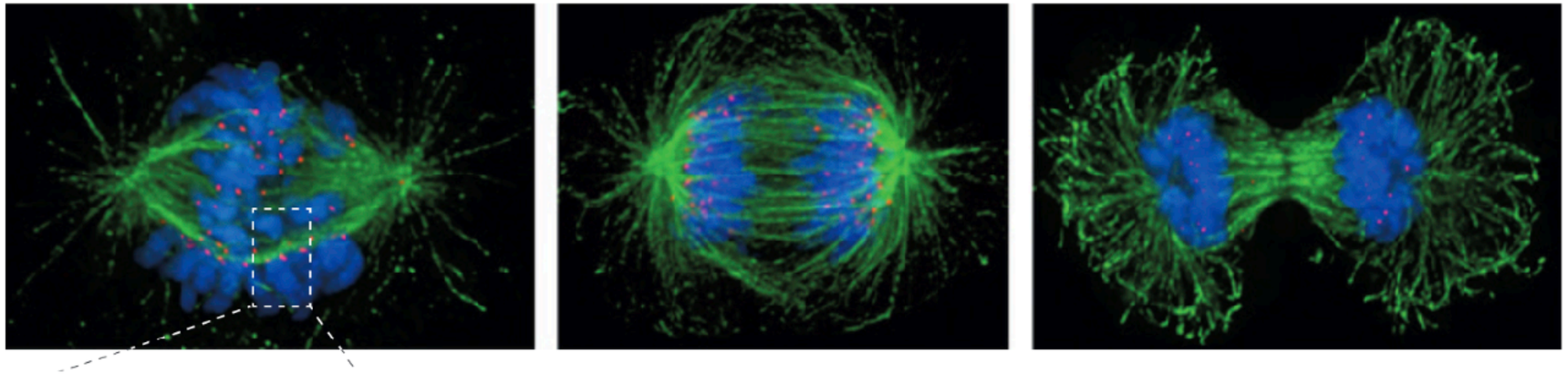




Kinetochores direct chromosome segregation during mitosis

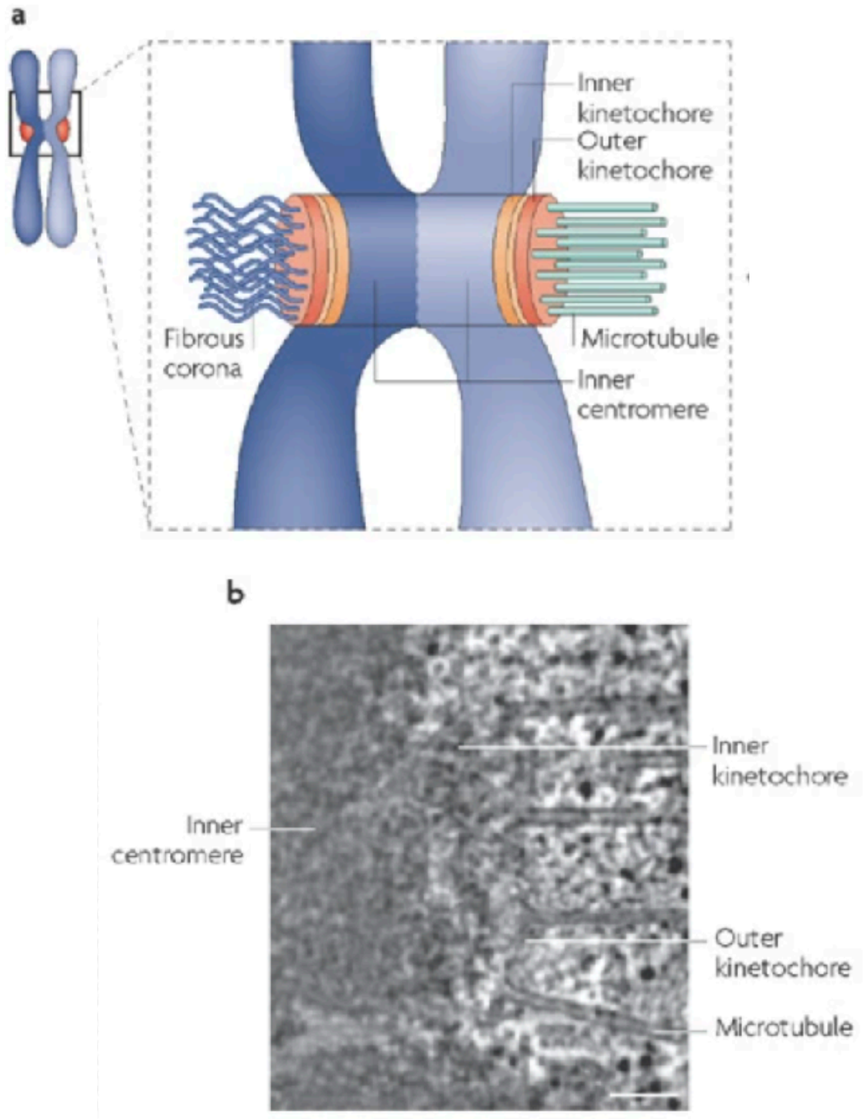


Images of mitotic Salamander cells hand-drawn by Walther Flemming in 1882.



Human mitotic cell imaged a few years ago
DNA- blue
Centromeres- red
Microtubules - green

The kinetochore directs chromosome segregation

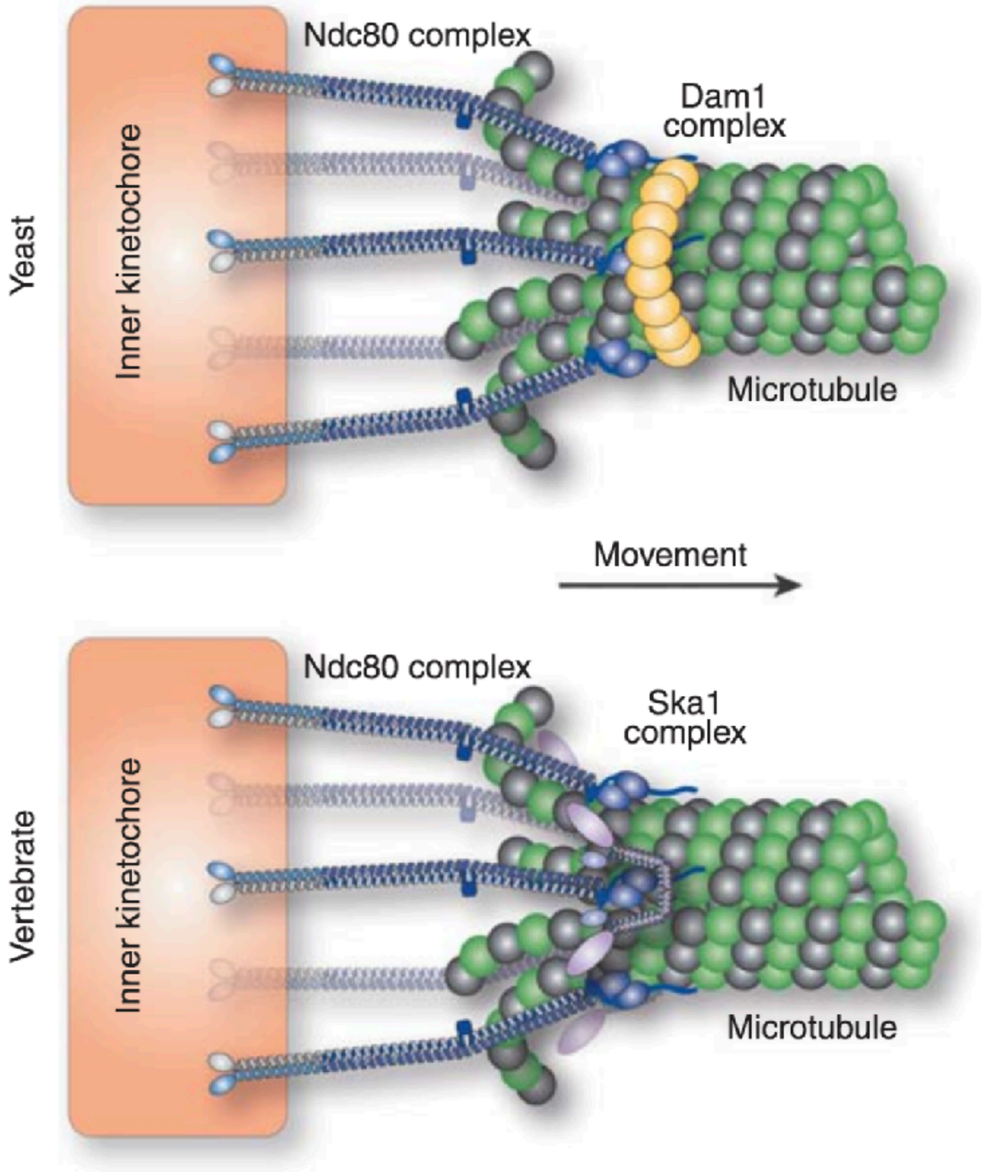


Protein complex that assembles on centromeric DNA

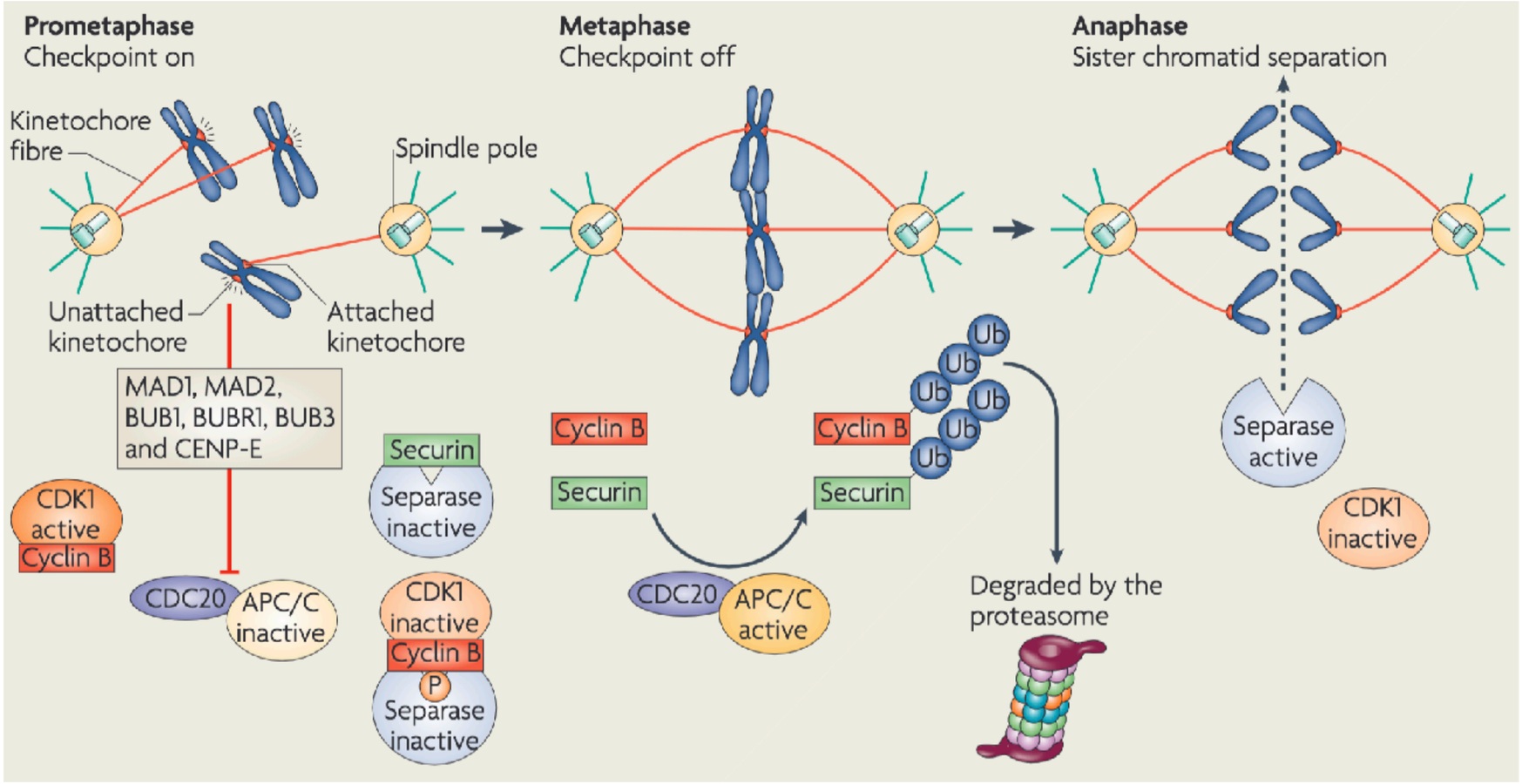
Microtubule attachment site

>100 unique components assemble in < 20 minutes

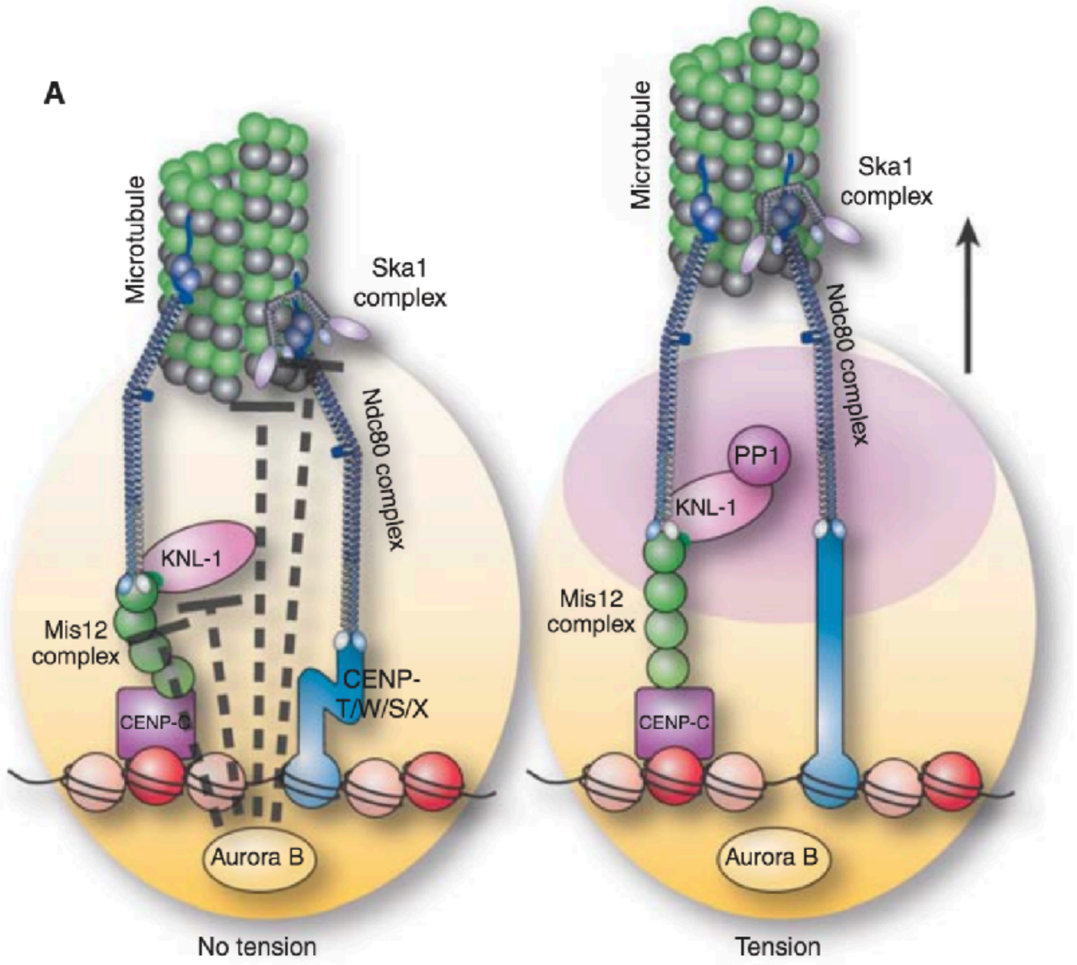
Kinetochores associate with depolymerizing microtubules



The spindle assembly checkpoint protects against aneuploidy

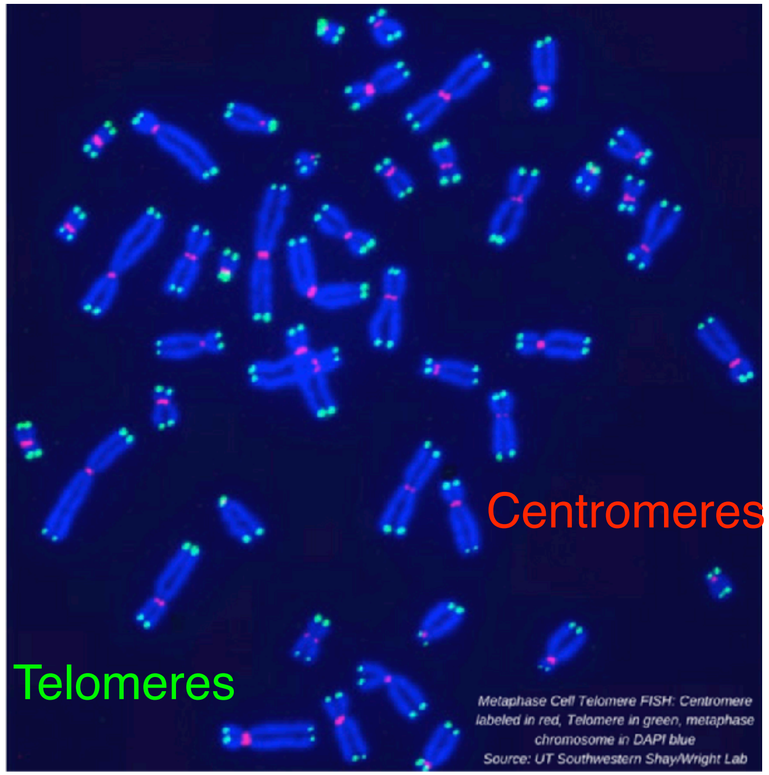


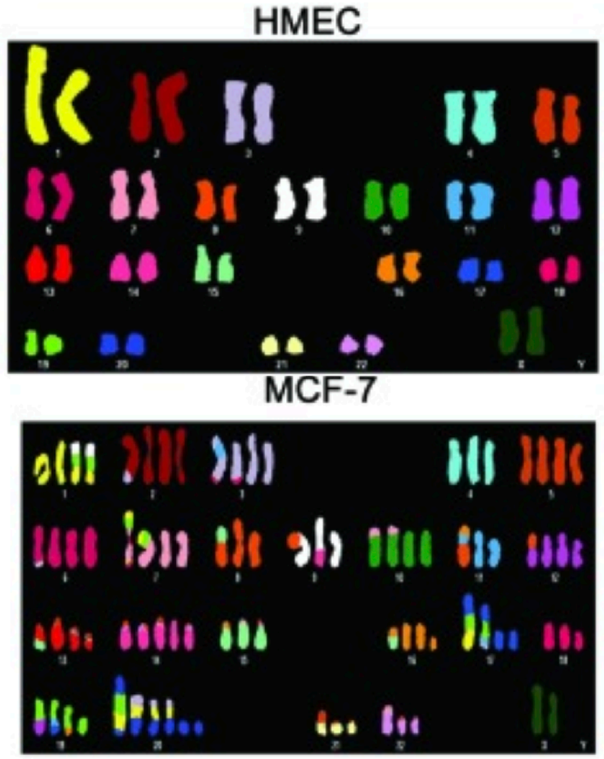
Aurora B regulates kinetochore-microtubule attachments



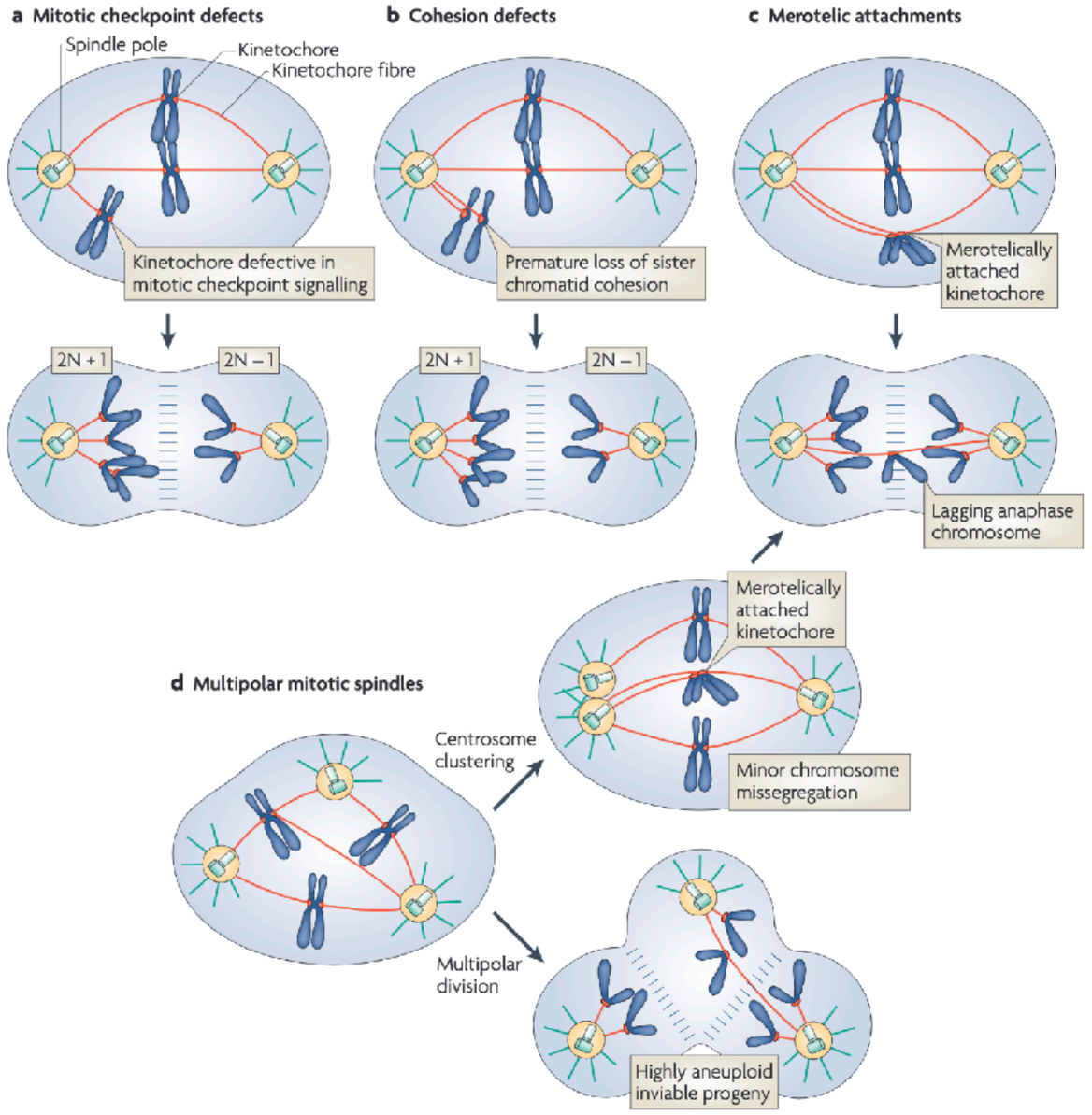
Why destabilize a tensionless kinetochore-microtubule attachment?

Chromosomal instability in cancer

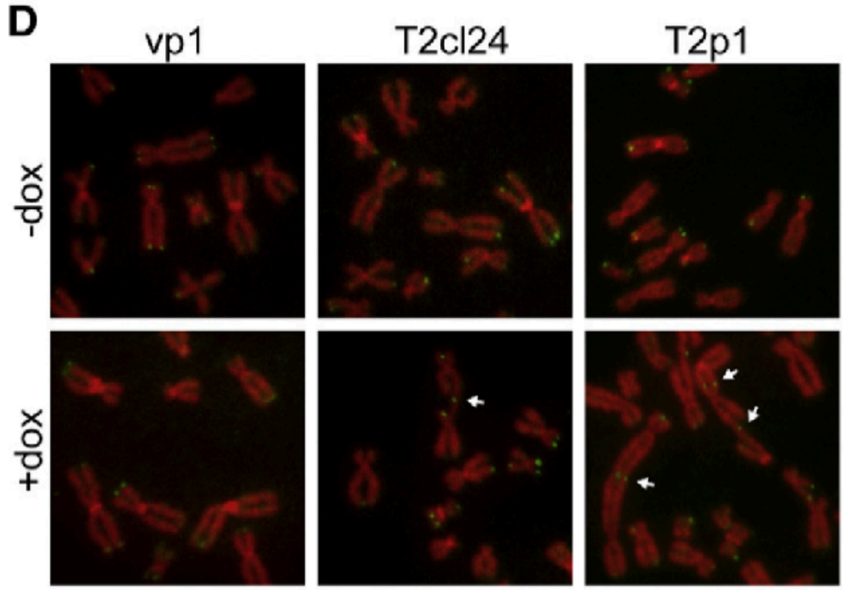


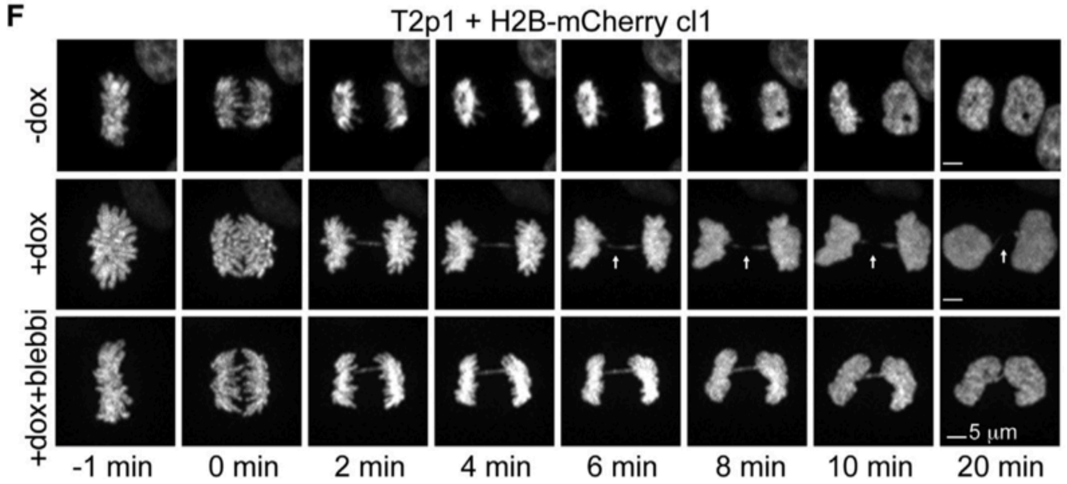


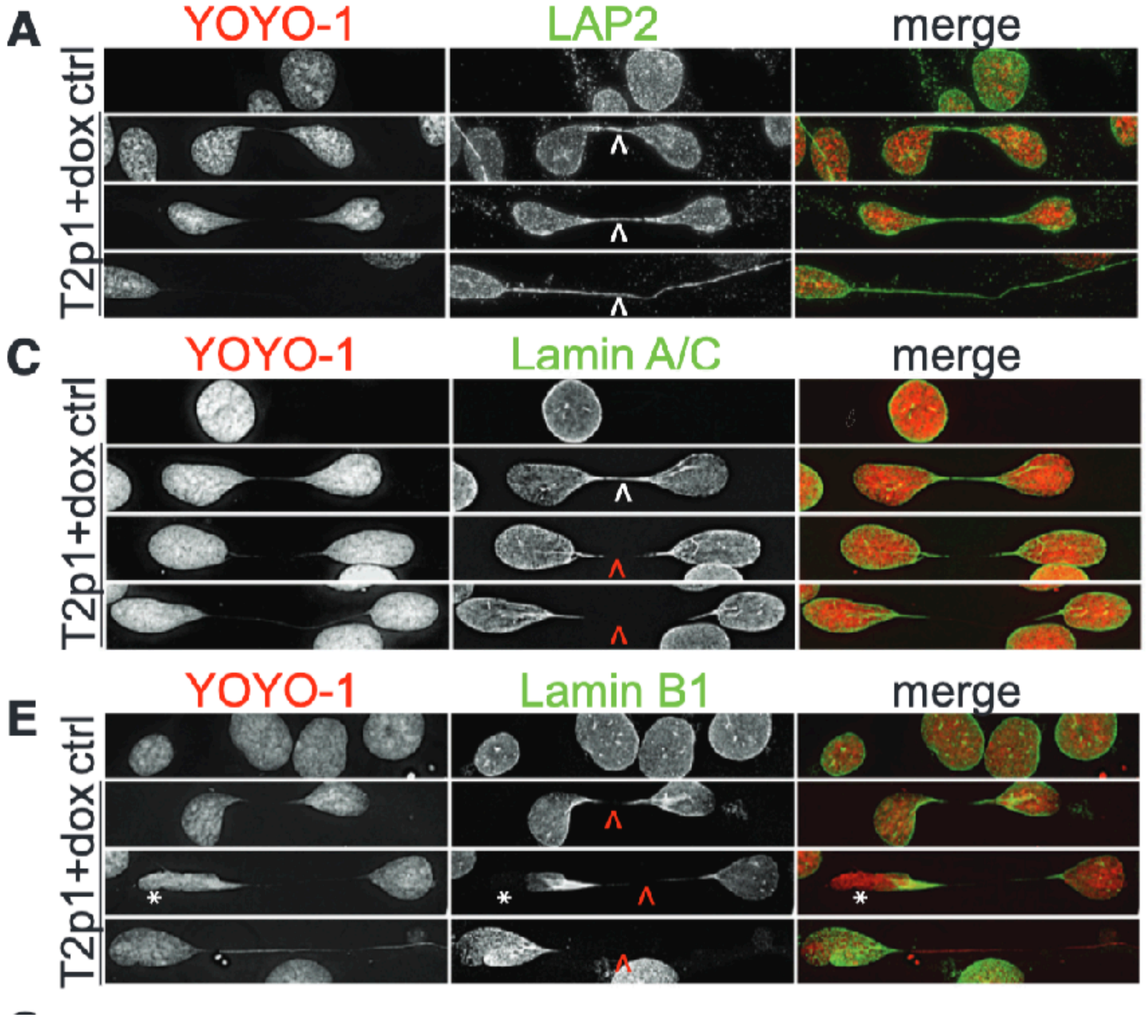
Pathways to aneuploidy



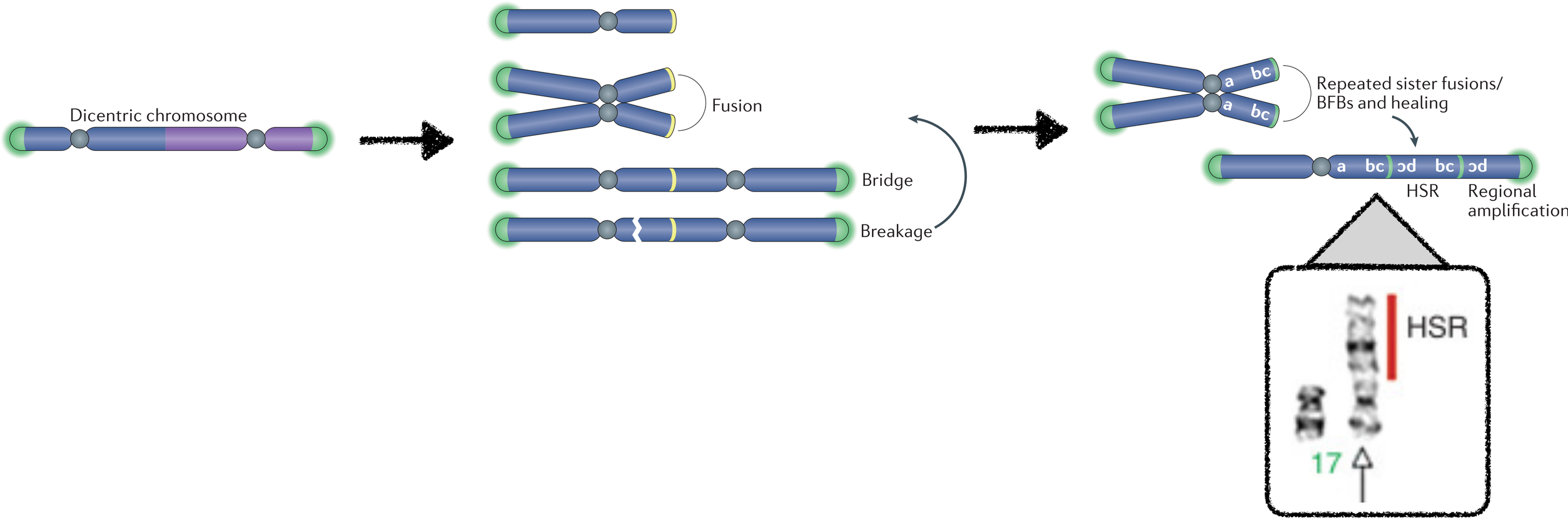
Dicentric chromosome segregation generates DNA bridges





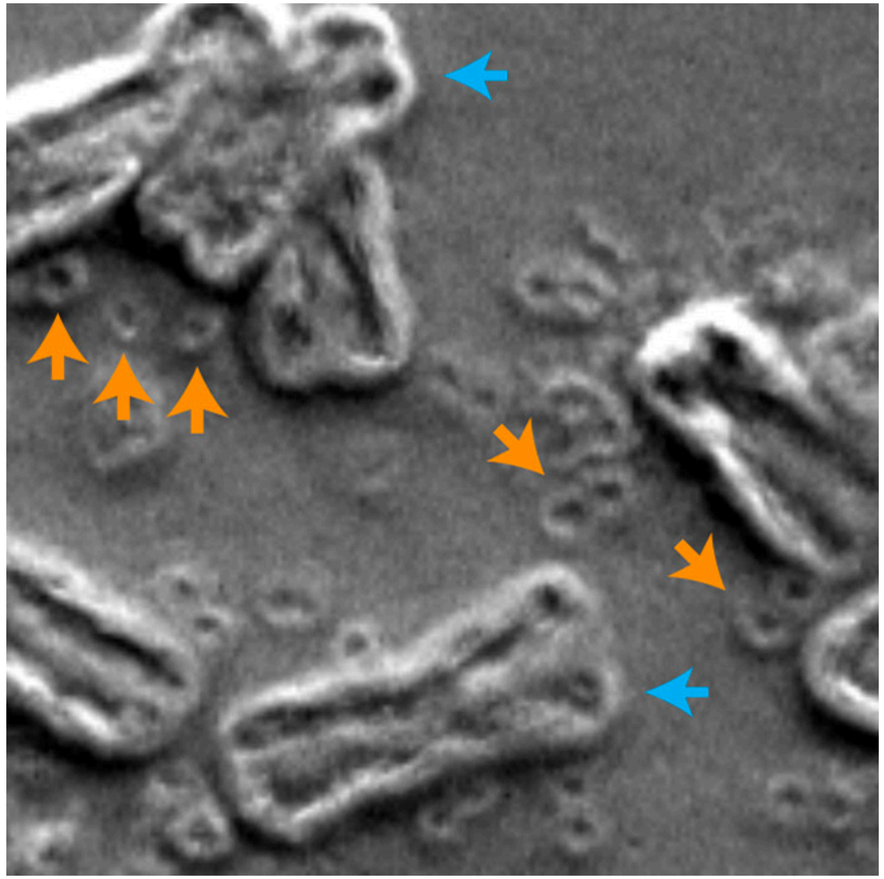


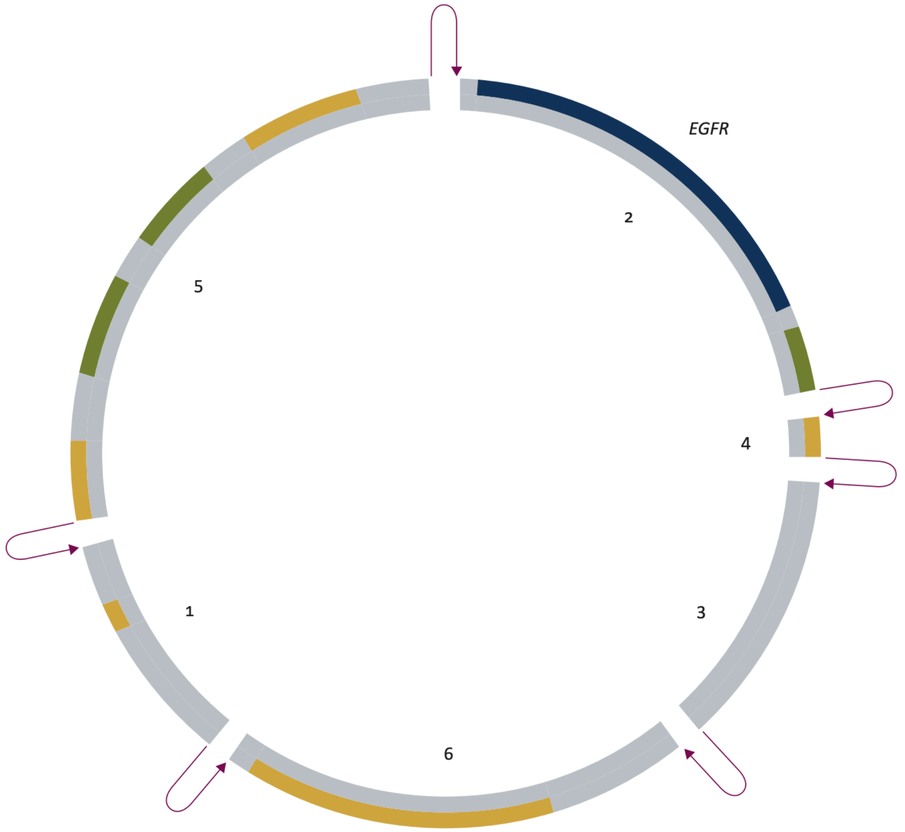
Telomere dysfunction can promote gene amplification & HSR formation



Maciejowski & de Lange, 2017 (PMID: 28096526); Li et al, 2014 (PMID: 24670643)

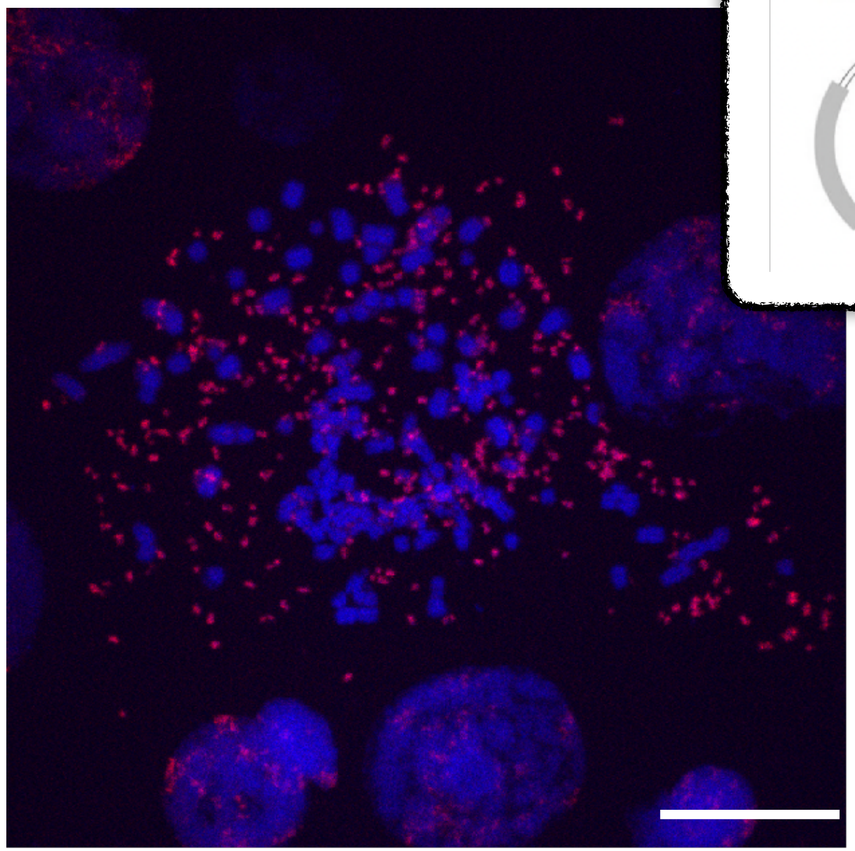
ecDNA are circular DNAs that lack centromeres & are frequently observed in cancer



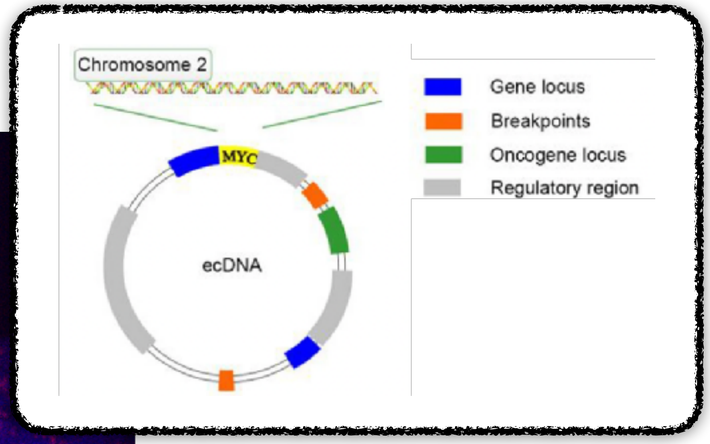


Extrachromosomal DNA (double minutes) are a common source of focal amplification

COLO320-DM



DNA *MYC*



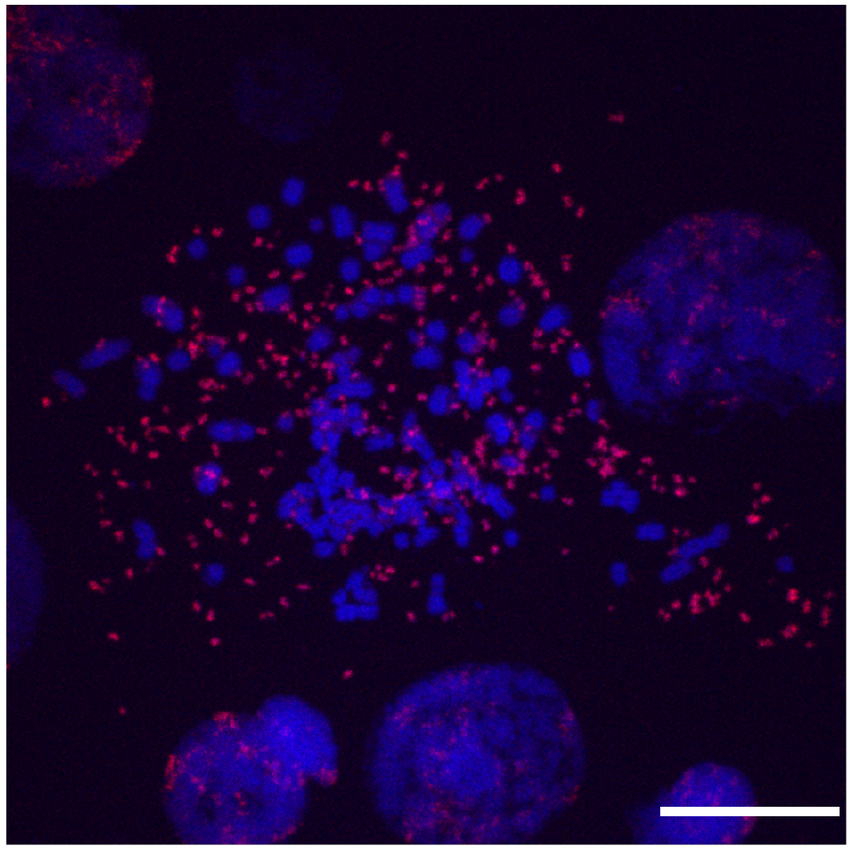
ecDNA are an emerging hallmark of cancer

- Increase oncogene copy number
- >60% of cancer types; ~14% of cancer genomes
- Enhanced transcription relative to linear amplifications
- Act as mobile enhancers

Kim et al, 2020 (PMID: 32807987); Wu et al, 2019 (PMID: 31748743); Zhu et al, 2021 (PMID: 33836152)

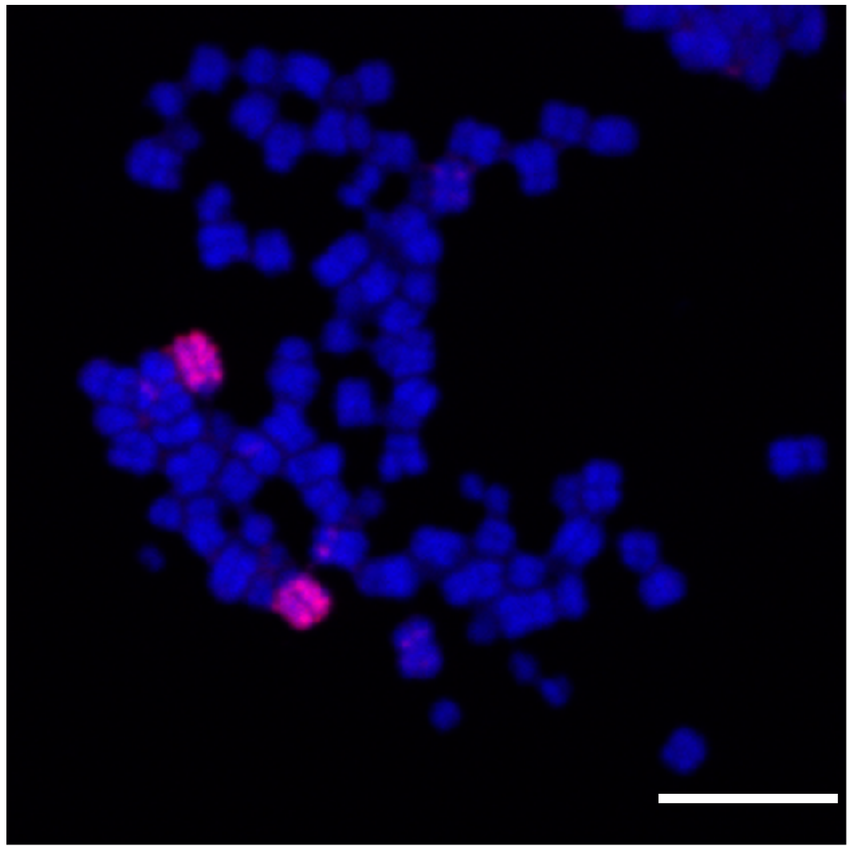
ecDNA can jump in and out of chromosomes to form HSRs

COLO320-DM



DNA *MYC*

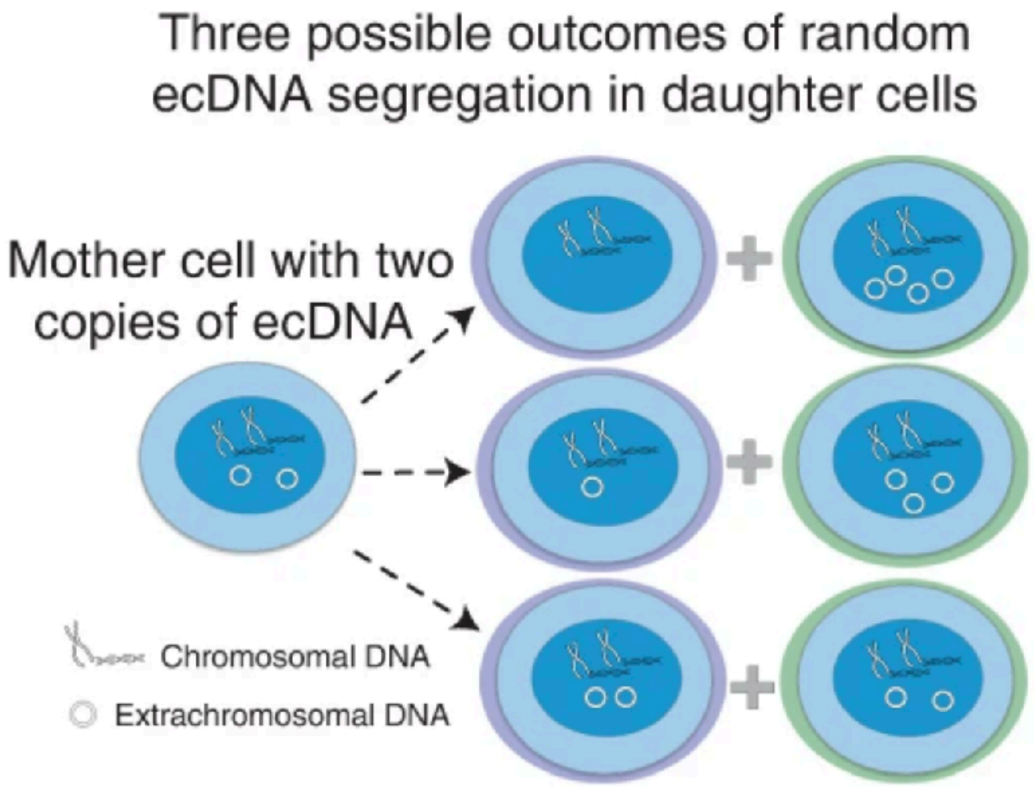
COLO320-HSR



DNA *MYC*

Benner et al, 1991 (PMID: 1720337); Nathanson et al, 2014 (PMID: 24310612)

Asymmetric ecDNA segregation results in rapid (& reversible) amplification



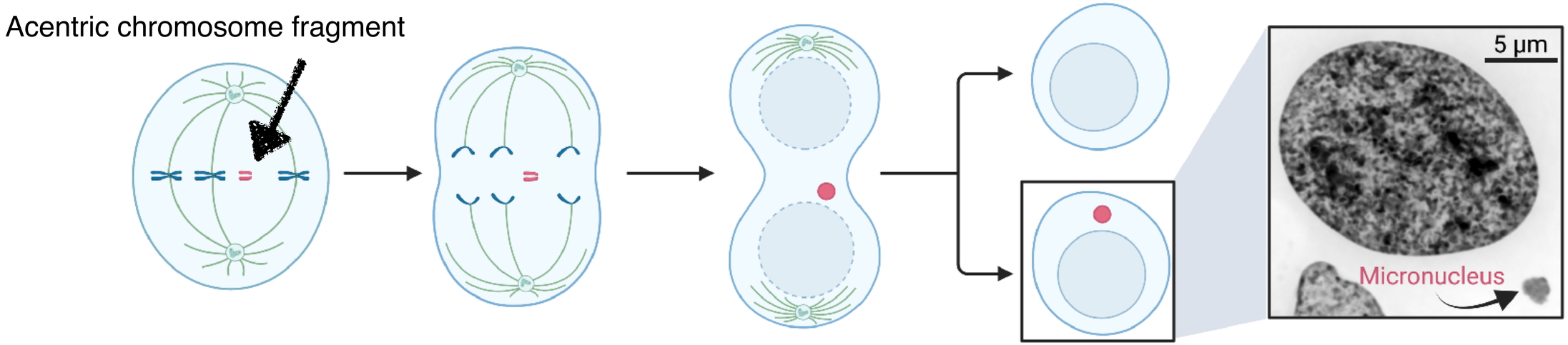
ecDNA can be segregated asymmetrically

- ecDNA lack centromeres
- Rapid accumulation over several cell divisions
- ecDNA replicate once per cell cycle

The mechanisms of mitotic ecDNA segregation are poorly understood

Lange et al, 2022 (PMID: 36123406); Kim et al, 2020 (PMID: 32807987); Wu et al, 2019 (PMID: 31748743); Zhu et al, 2021 (PMID: 33836152)

Acentric chromosomes/chromosome fragments frequently form micronuclei



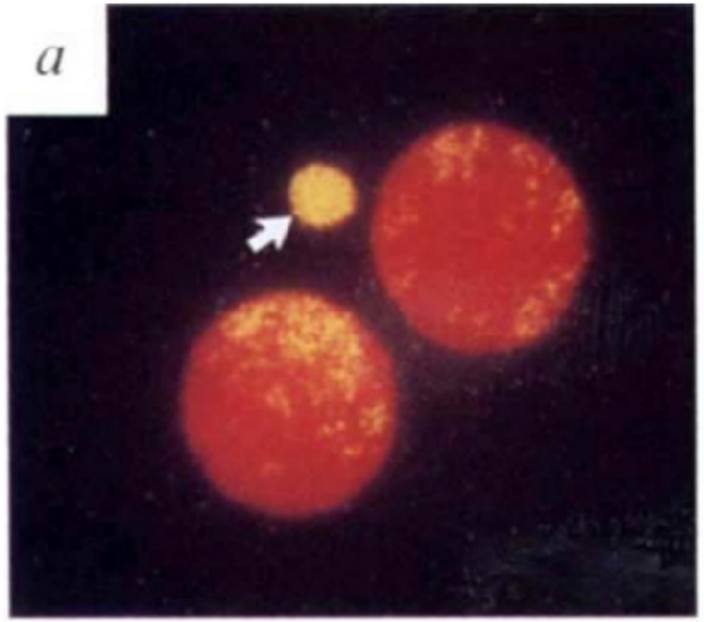
Micronucleation causes broad dysfunction

- DNA damage
- Transcriptional silencing
- DNA replication defects
- Innate immune activation

Hatch et al., 2013 (PMID: 23827264); Maciejowski & Hatch, 2020 (PMID: 32692592); Maciejowski et al., 2015 (PMID: 26687355)
Crasta et al., 2012 (PMID: 22258507); Zhang et al., 2015 (PMID: 26017310); Agustinus et al., 2022 (https://doi.org/10.1101/2022.01.12.475944)

ecDNA may form micronuclei after mis-segregating as large clusters

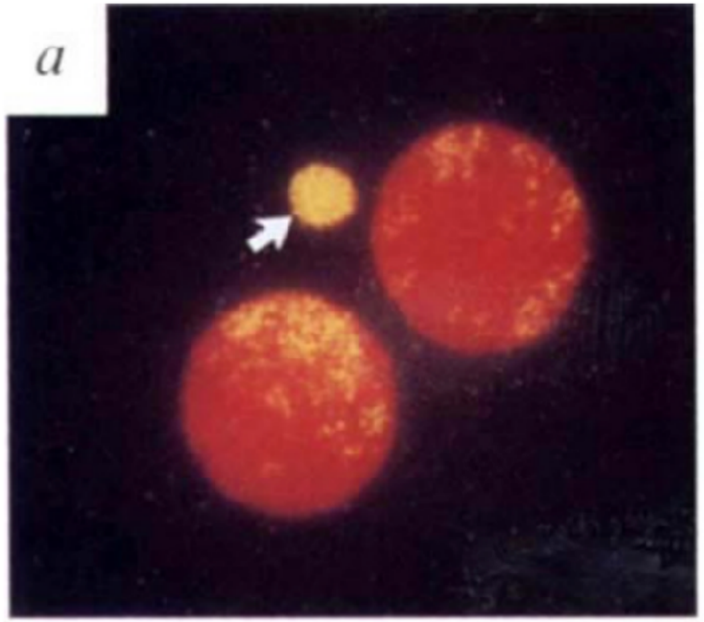
ecDNA+ micronuclei



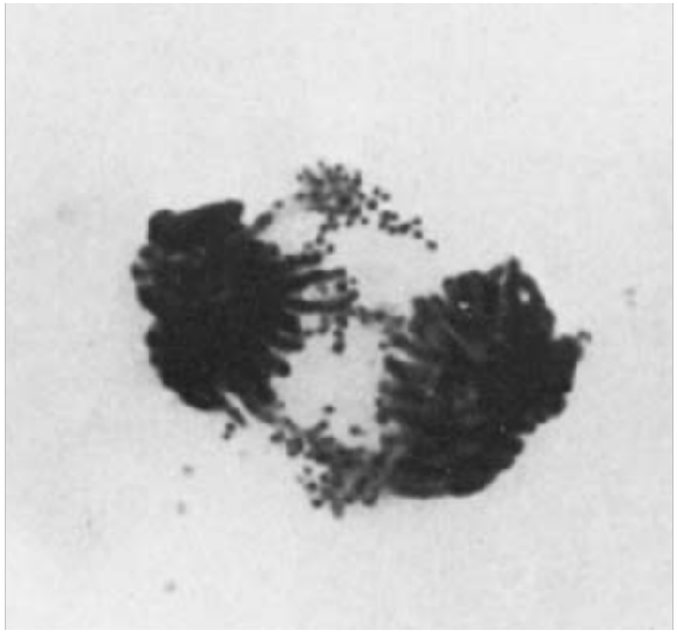
Shimizu et al, 1996 (PMID: 8528254)

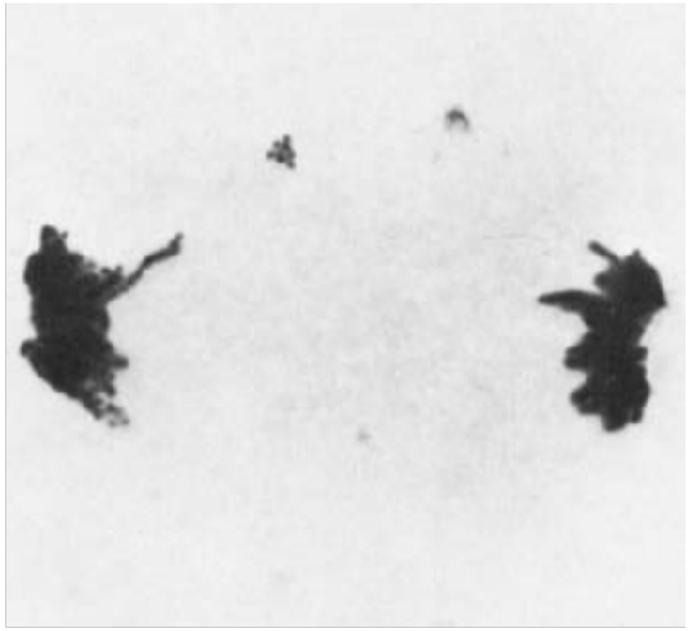
ecDNA may segregate by tethering to mitotic chromosomes

ecDNA+ micronuclei



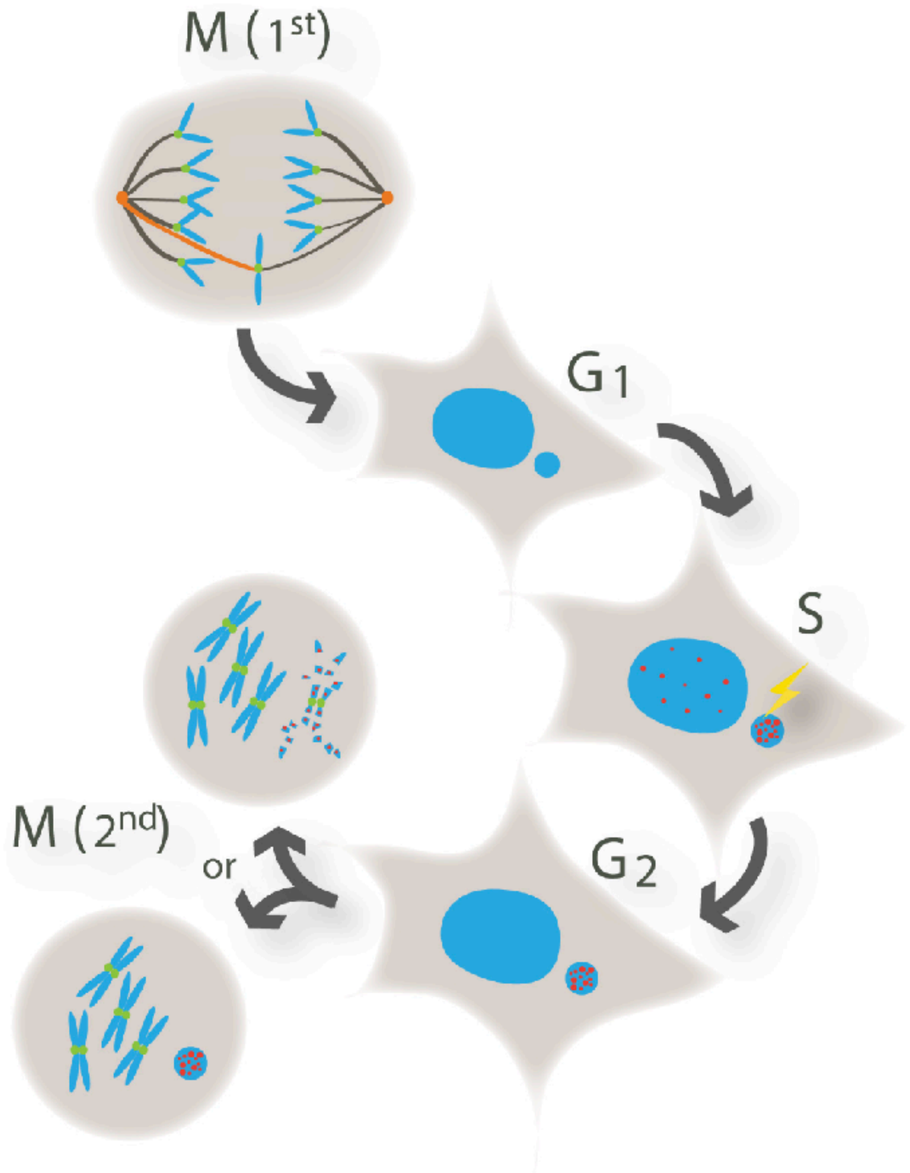
ecDNA segregate via chromosome ‘hitchhiking’

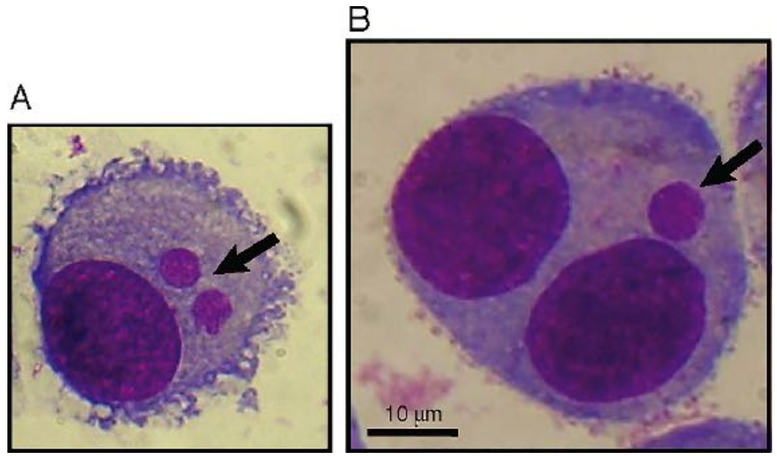




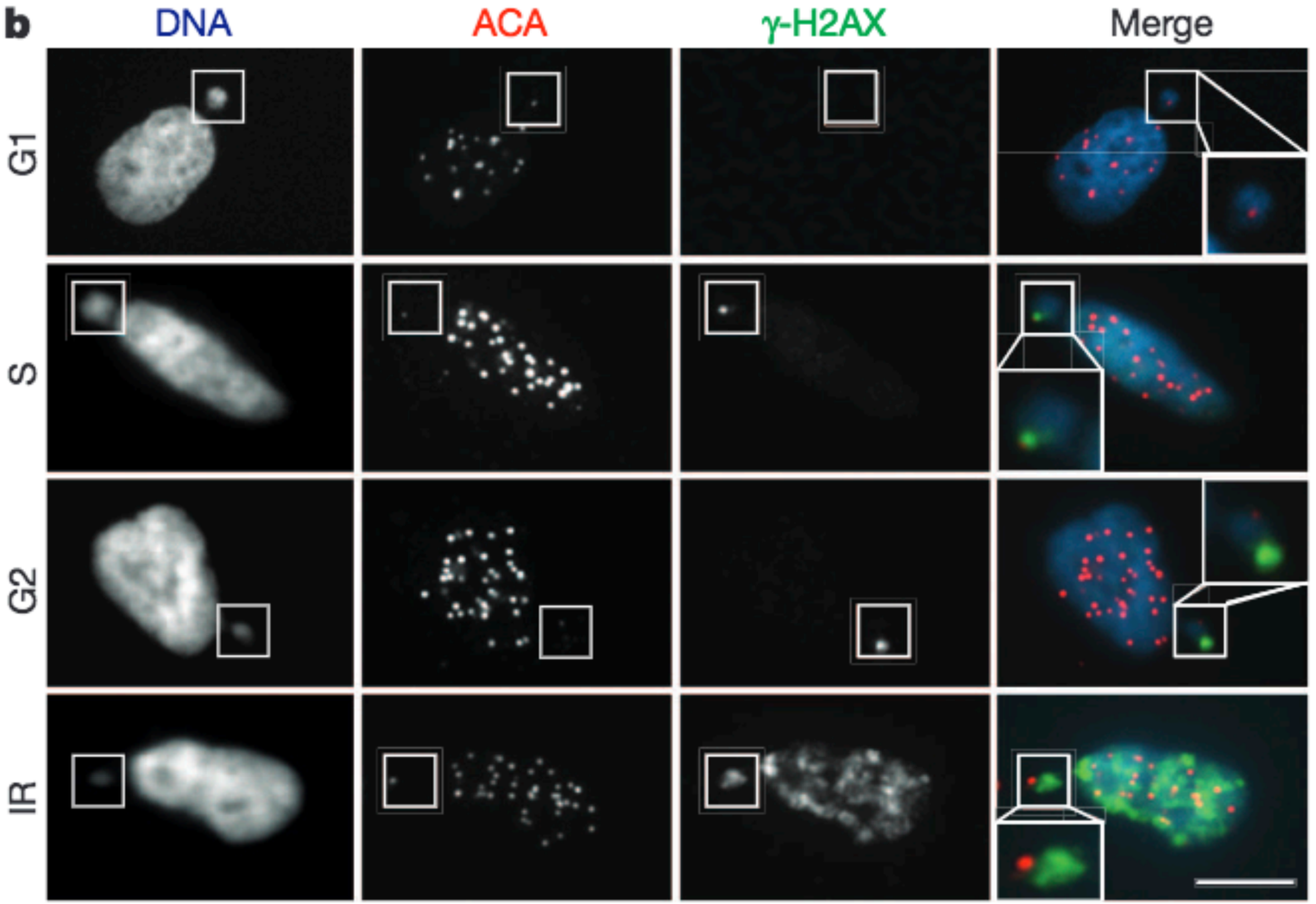
Shimizu et al, 1996 (PMID: 8528254); Levan & Levan, 1978 (PMID: 649427); Kanda et al, 2001 (PMID: 11112689); Barker & Hsu, 1978 (PMID: 299656)

CIN generates micronuclei

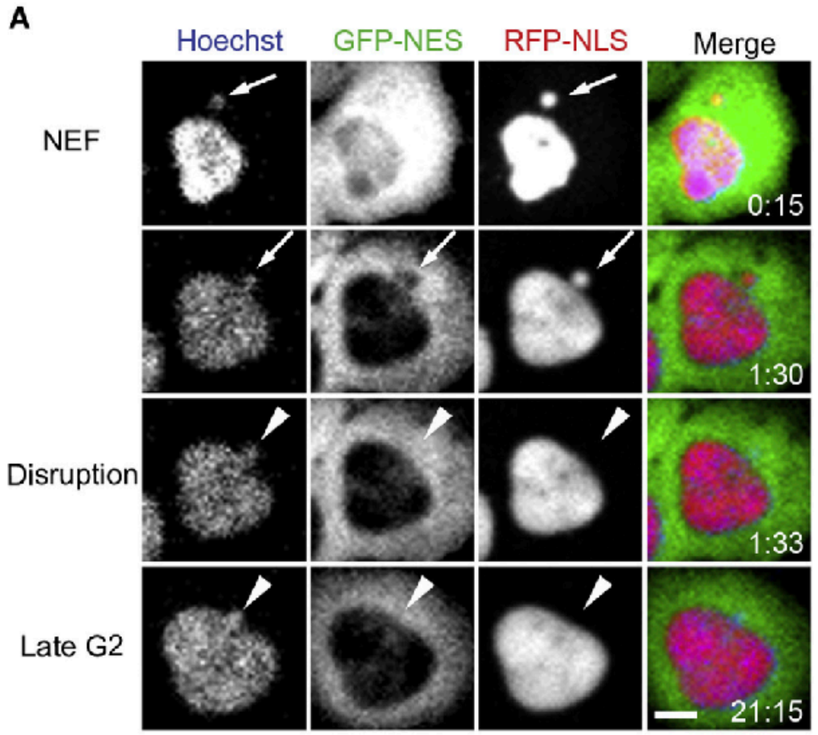




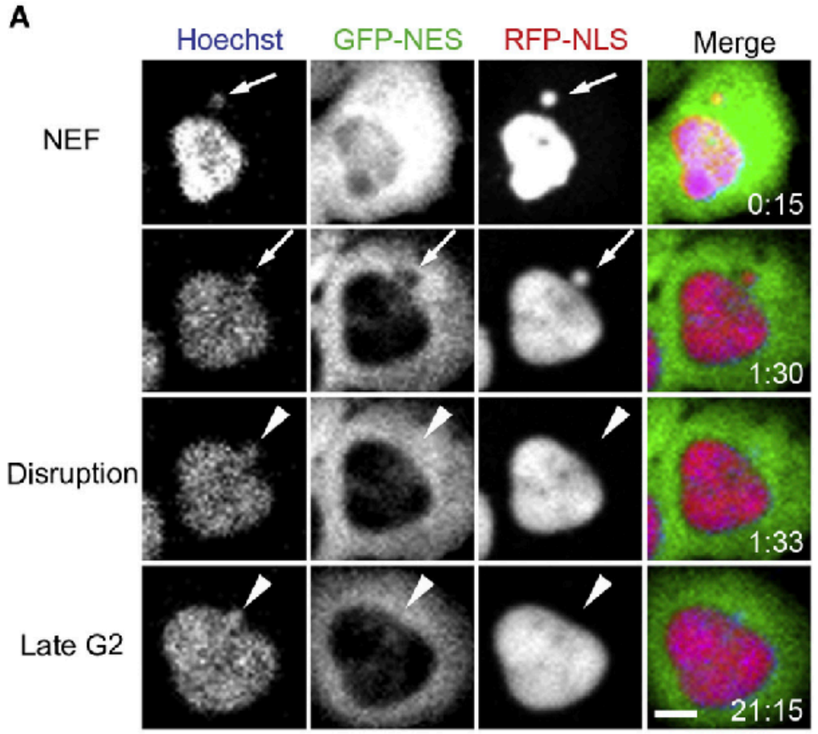
Micronuclei exhibit evidence of DNA damage

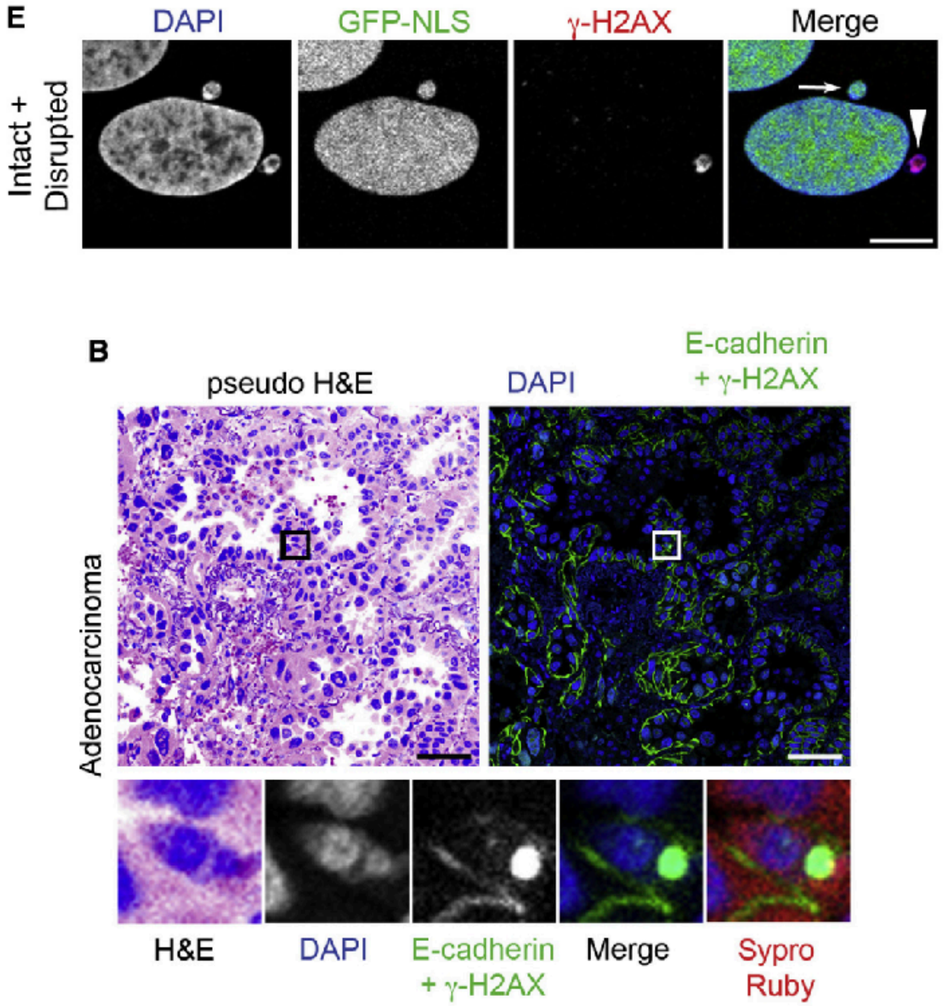


Micronuclei lose nuclear compartmentalization

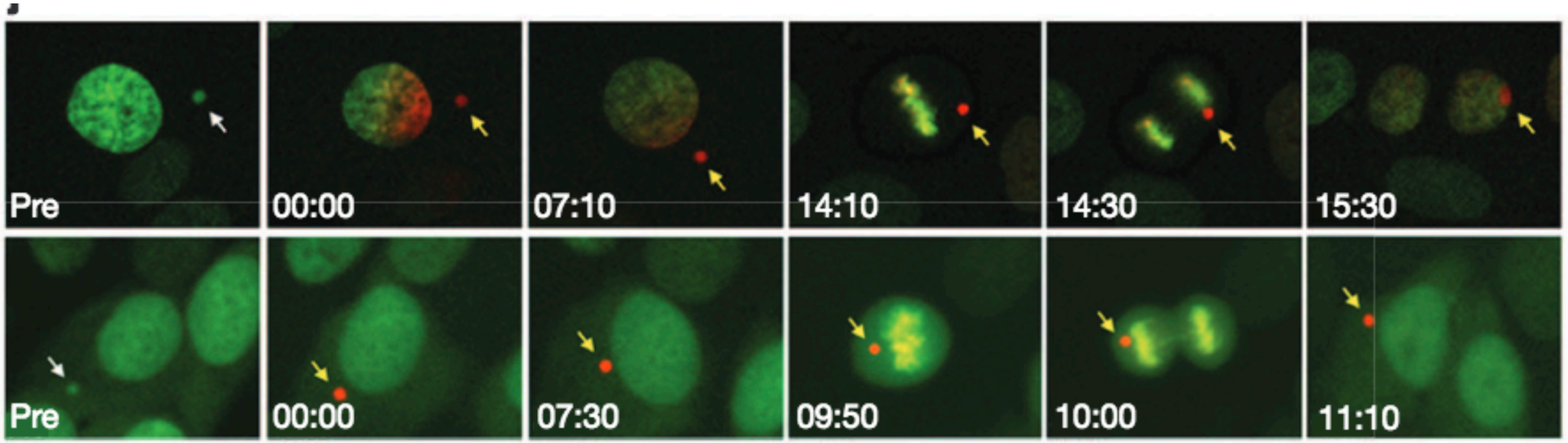


Micronuclei lose nuclear compartmentalization

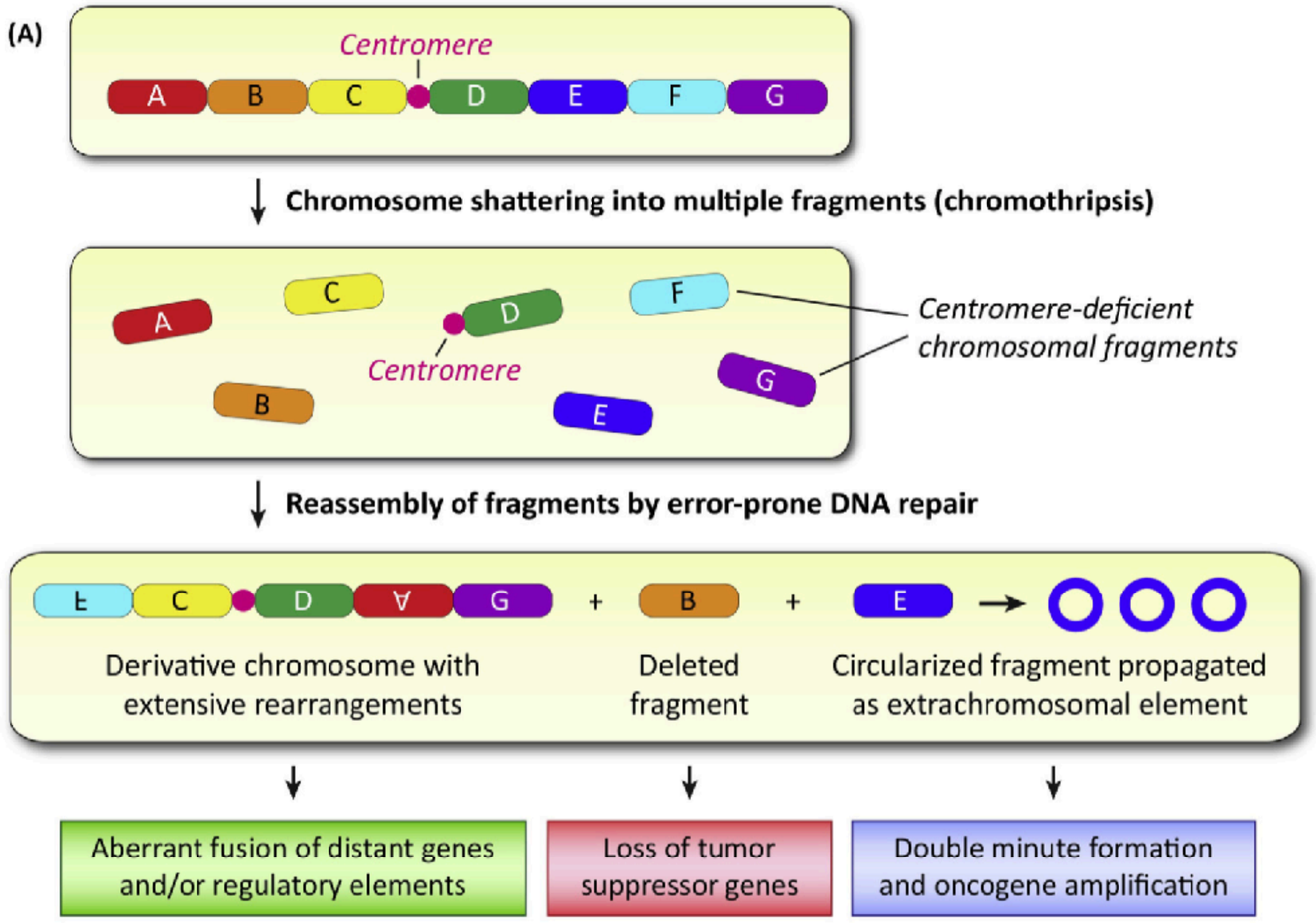


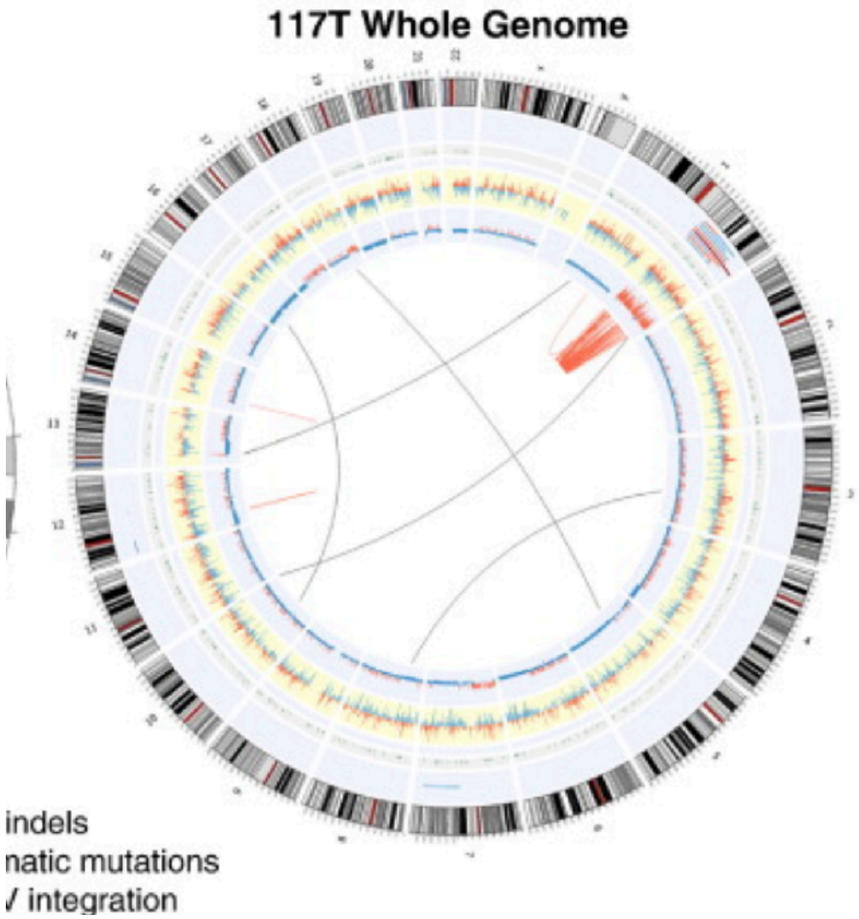


Micronuclei can re-enter main genome after mitotic nuclear envelope breakdown



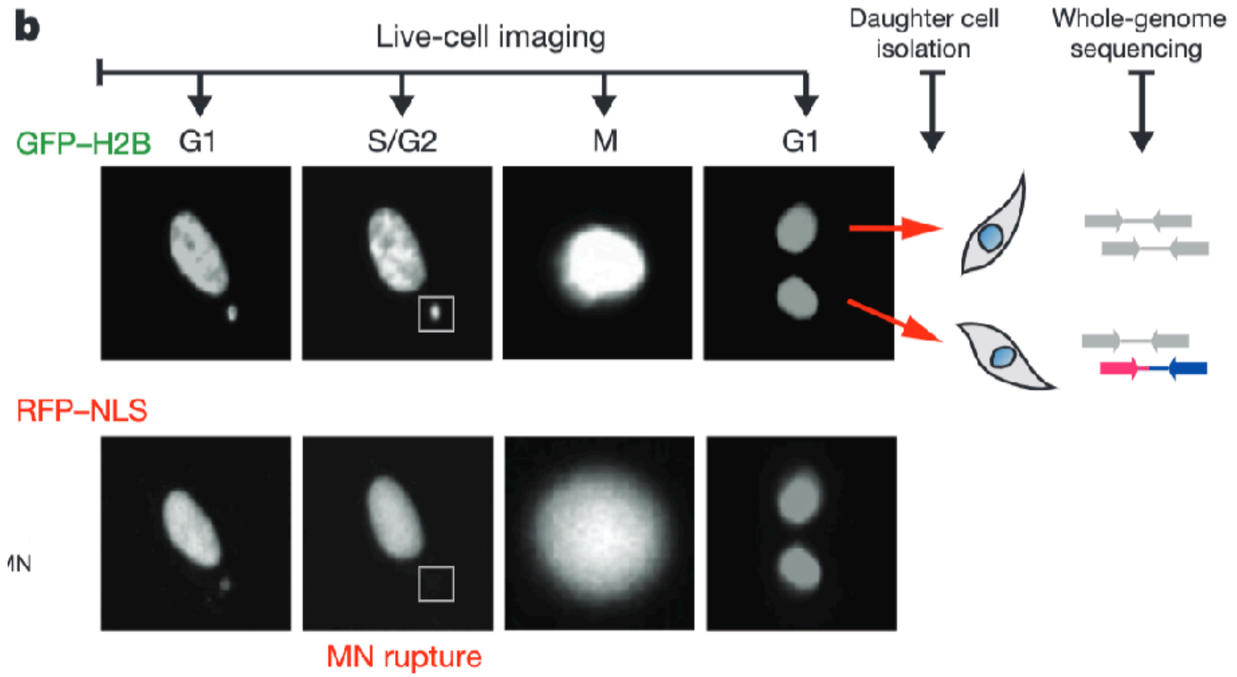
Does micronucleation lead to chromothripsis?

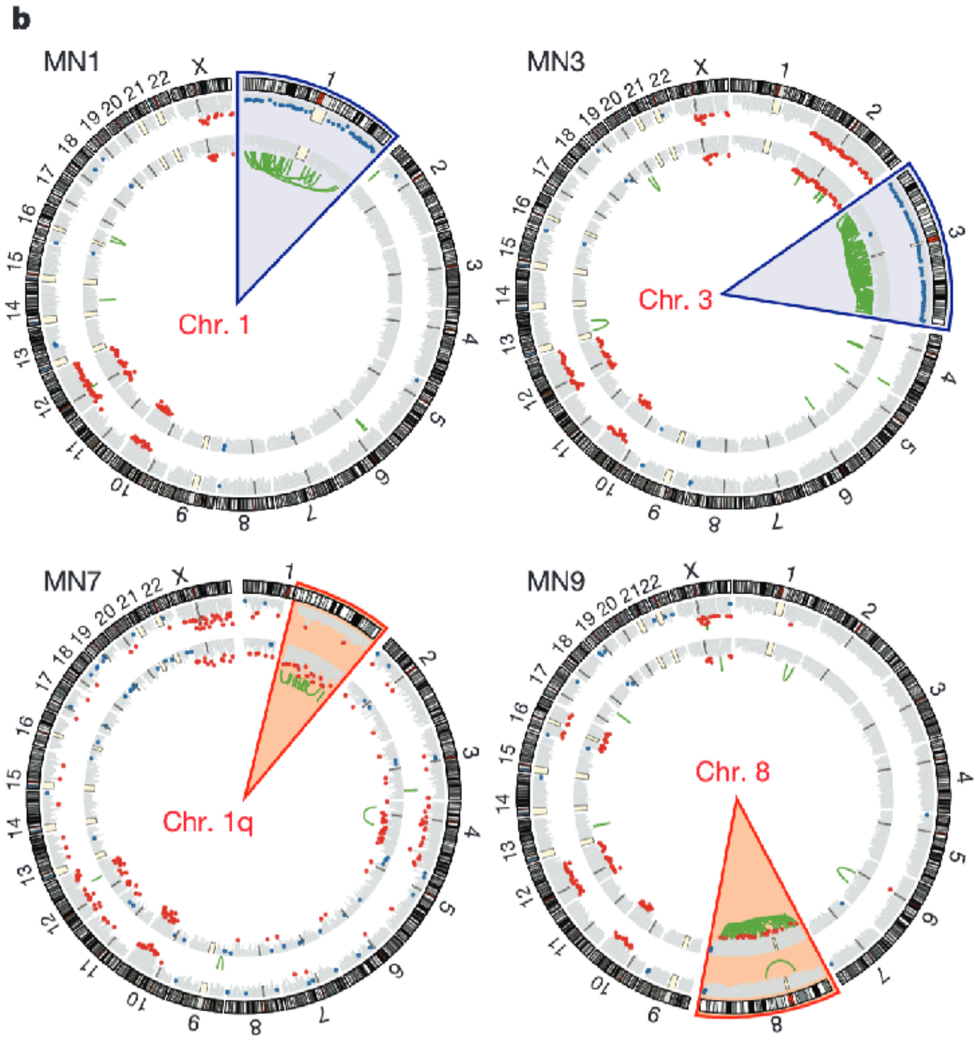




Ly & Cleveland, *Trends in Cell Biology*, 2015.

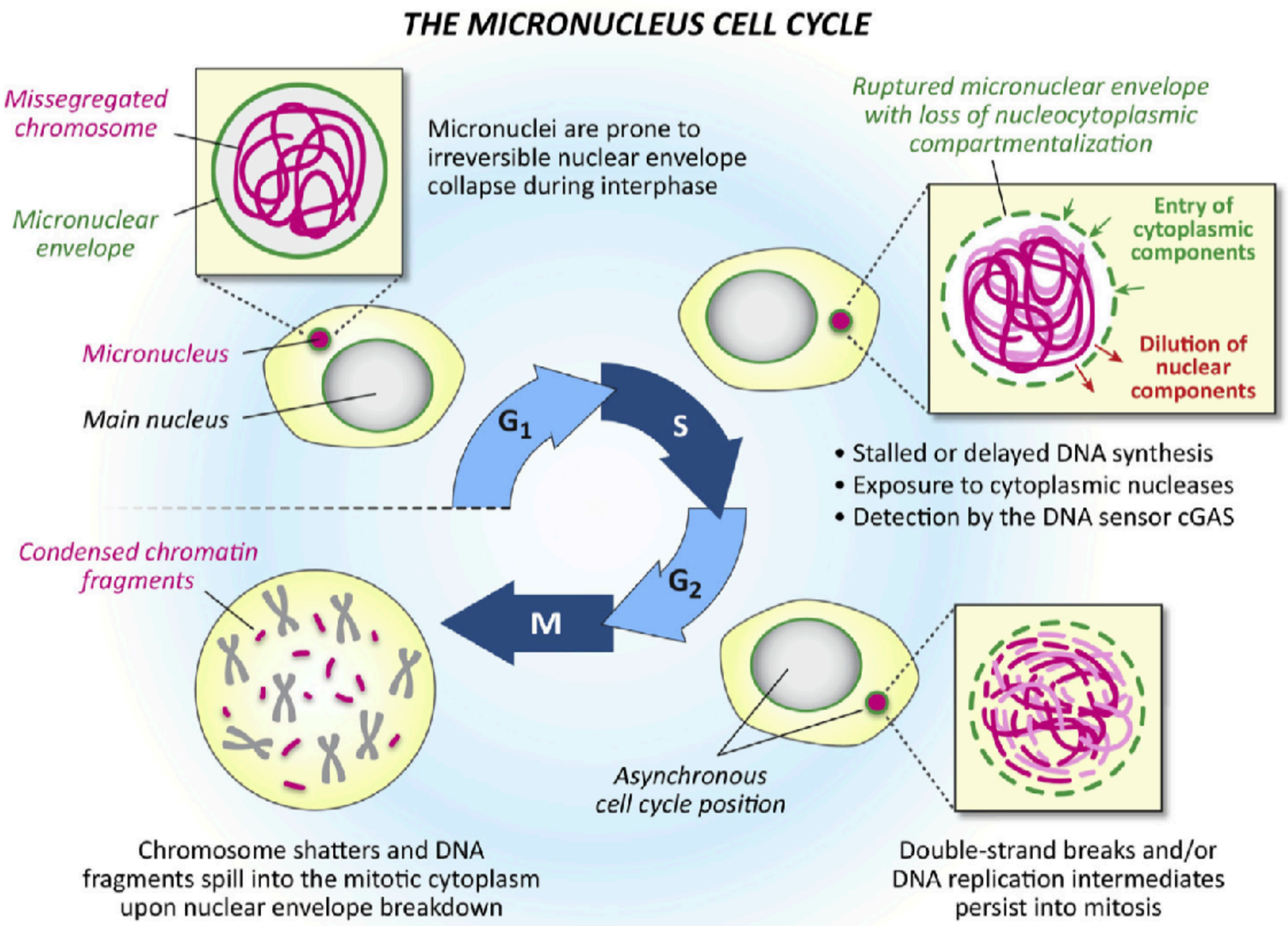
Does micronucleation lead to chromothripsis?





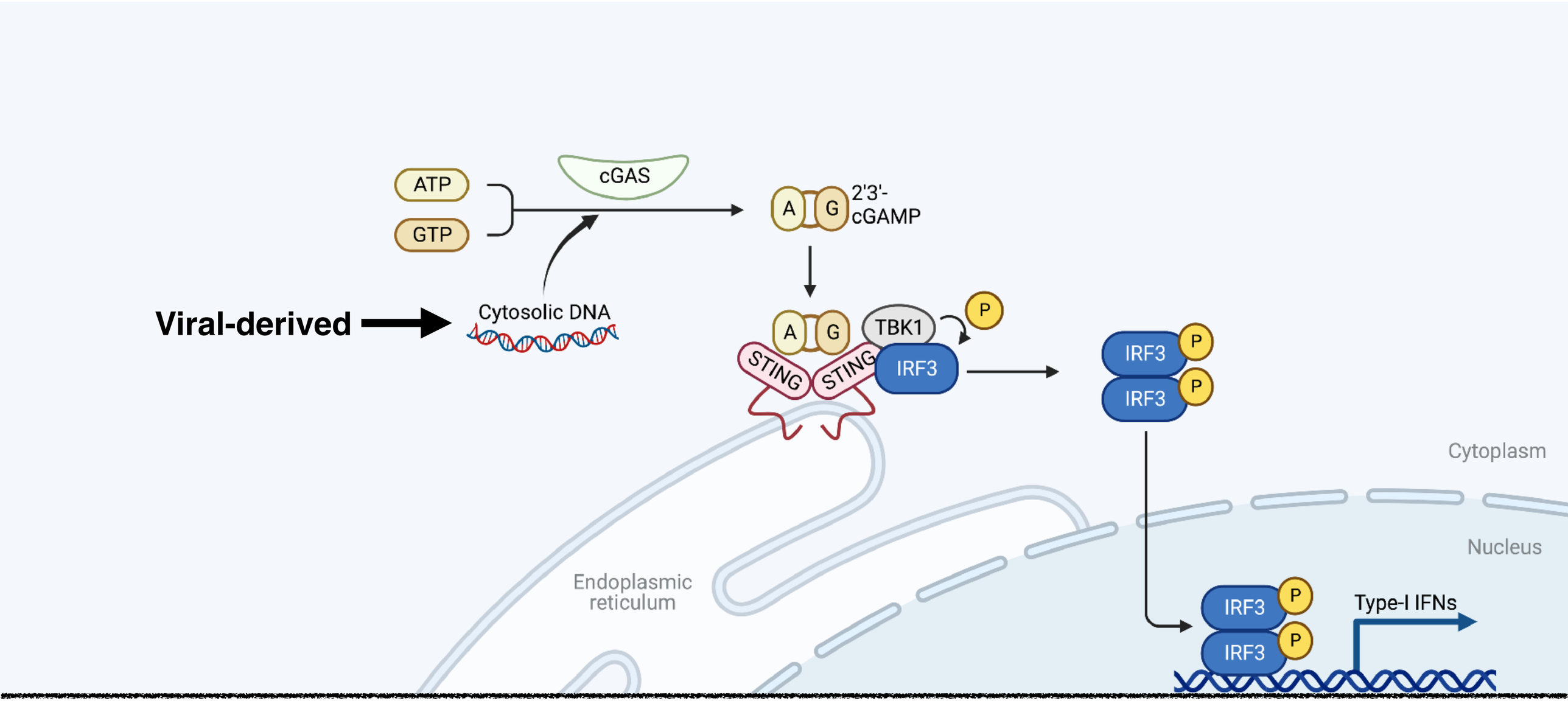
Zhang et al., *Nature*, 2015.

DNA damage in micronuclei triggers catastrophic shattering of chromosomes



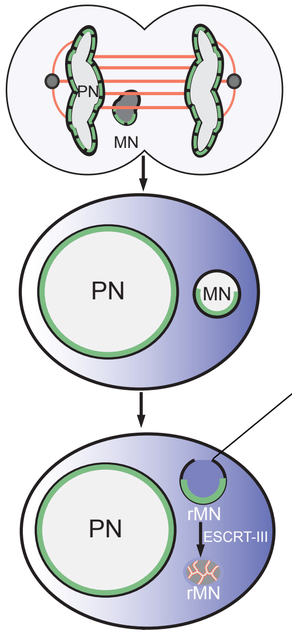
Ly & Cleveland, *Trends in Cell Biology*, 2015.

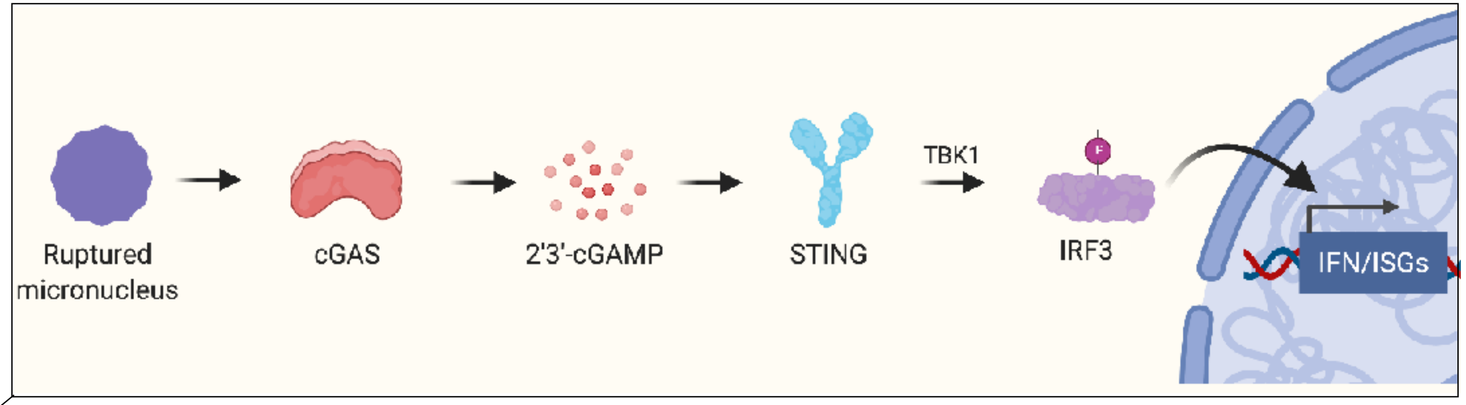
The cGAS-STING pathway initiates a pro-inflammatory response to foreign DNA

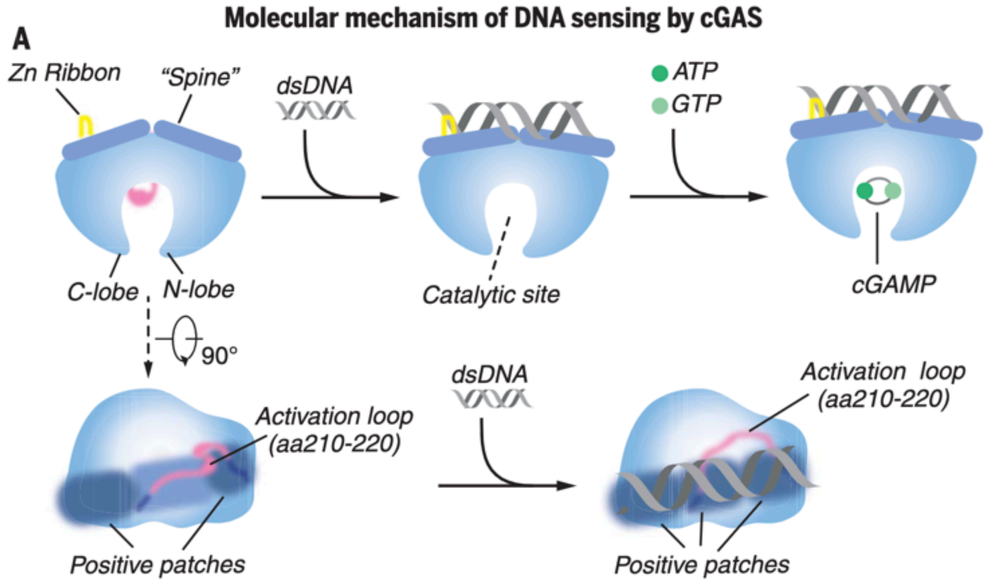


Sun et al., 2013 (PMID: 23258413); Ablasser et al., 2013 (PMID: 23722158); Gao et al., 2013 (PMID: 23647843); Gao et al., 2013 (PMID: 23910378); Ishikawa & Barber, 2008 (PMID: 18724357); Ishikawa et al., 2009 (PMID: 19776740); Kranzusch et al., 2013 (PMID: 23707061)

Can NE rupturing activate an immune response?

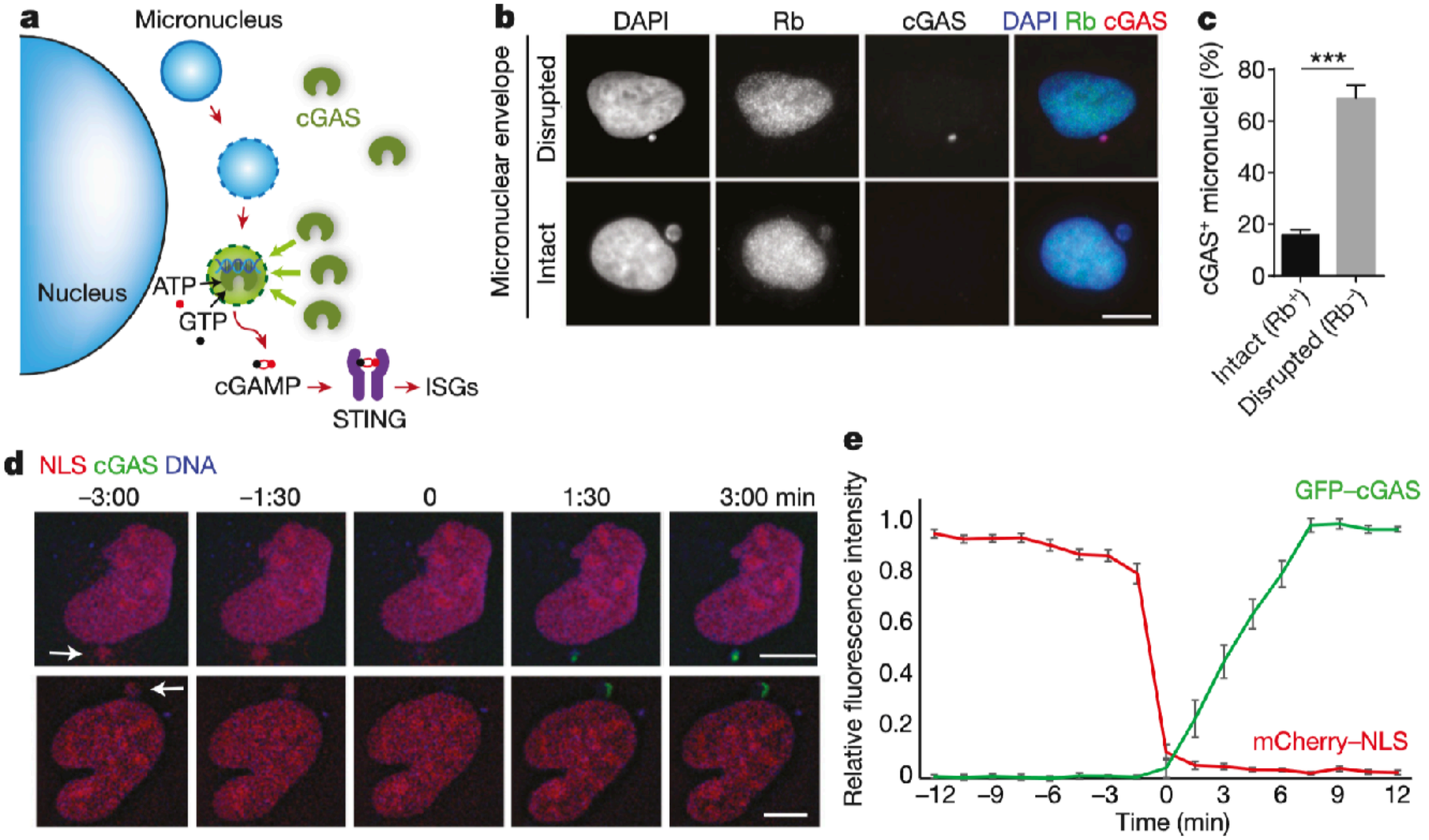




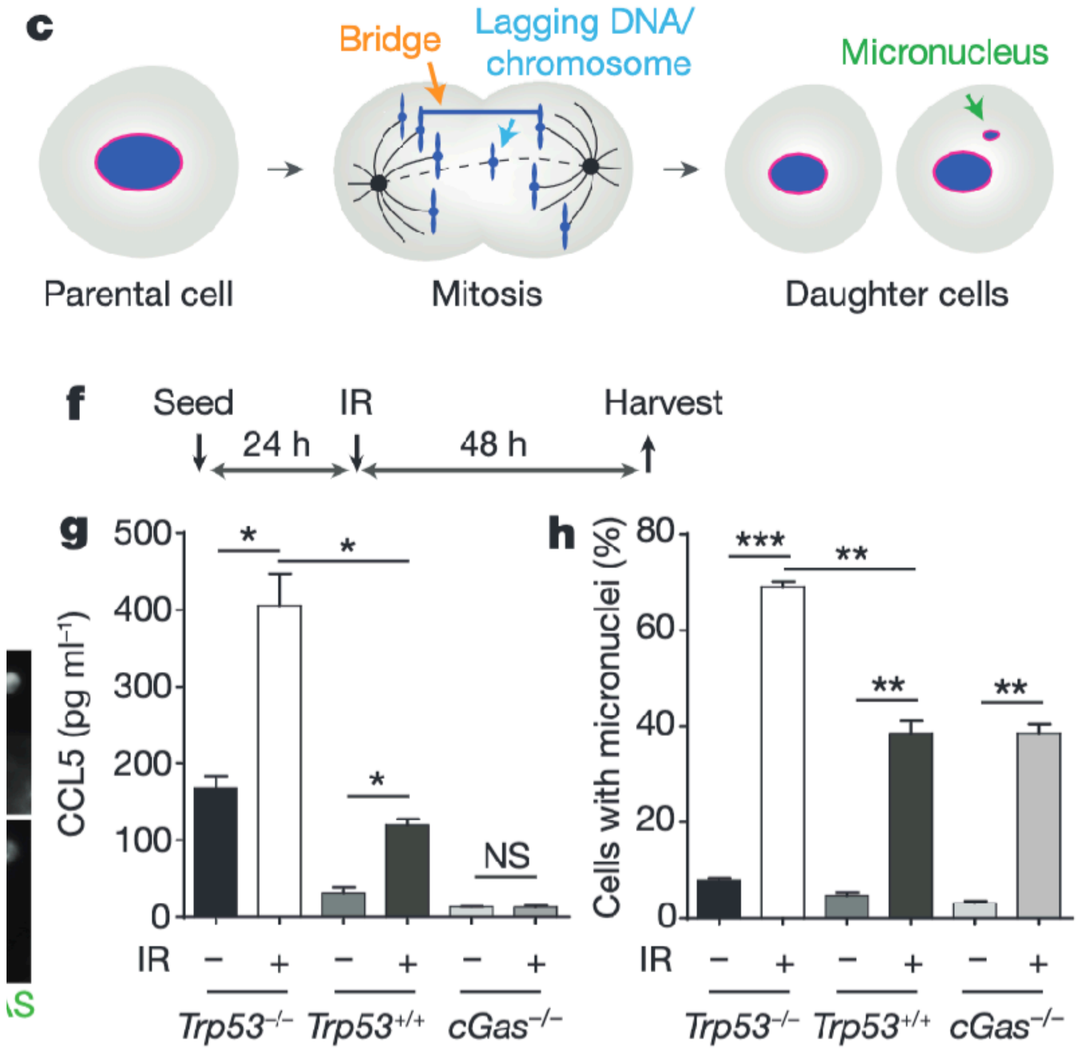


How could you test if micronuclei activate the cGAS/STING pathway?

cGAS localizes to micronuclei upon micronuclear envelope rupture



ISG upregulation associates with presence of micronuclei



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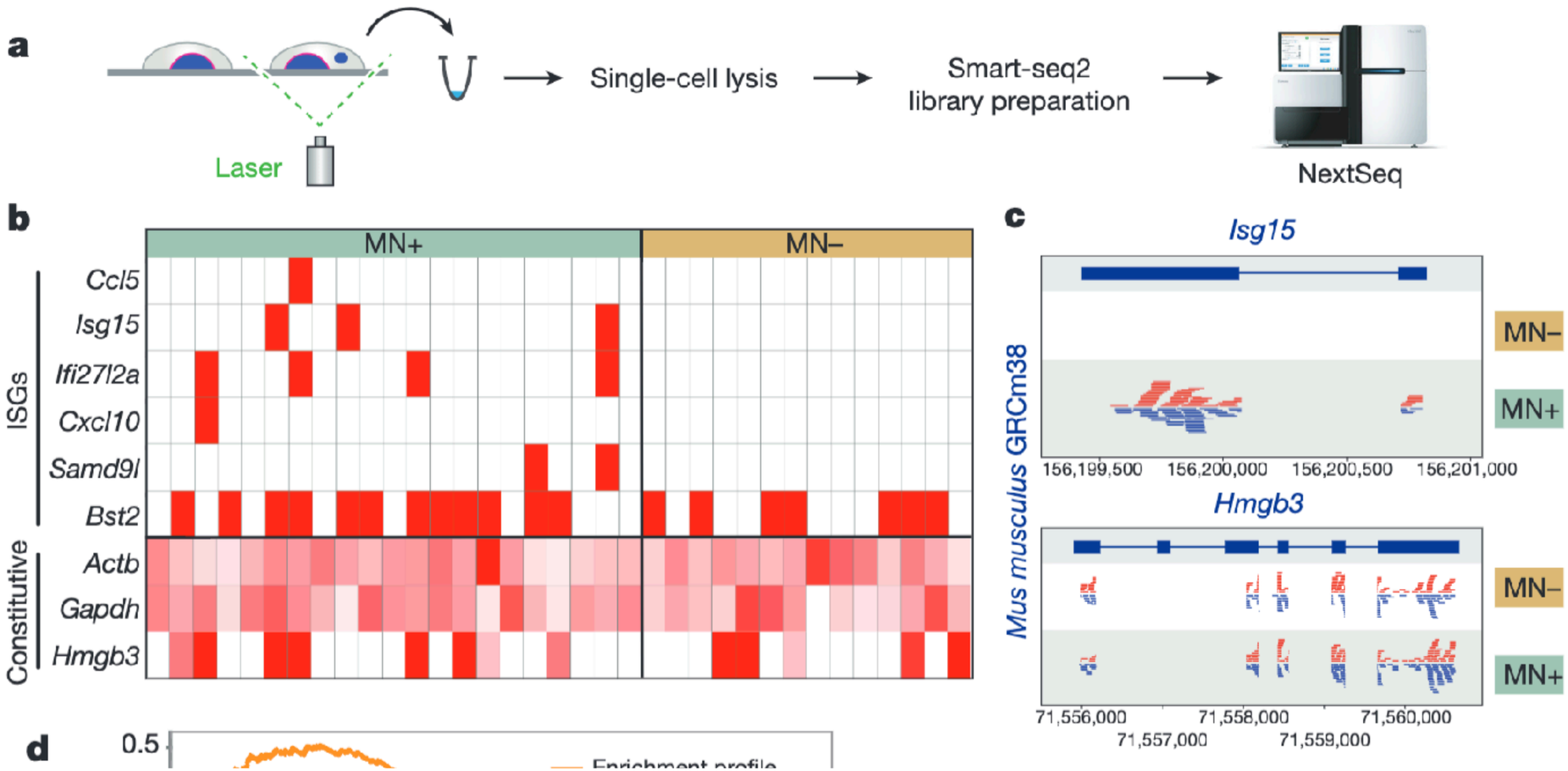
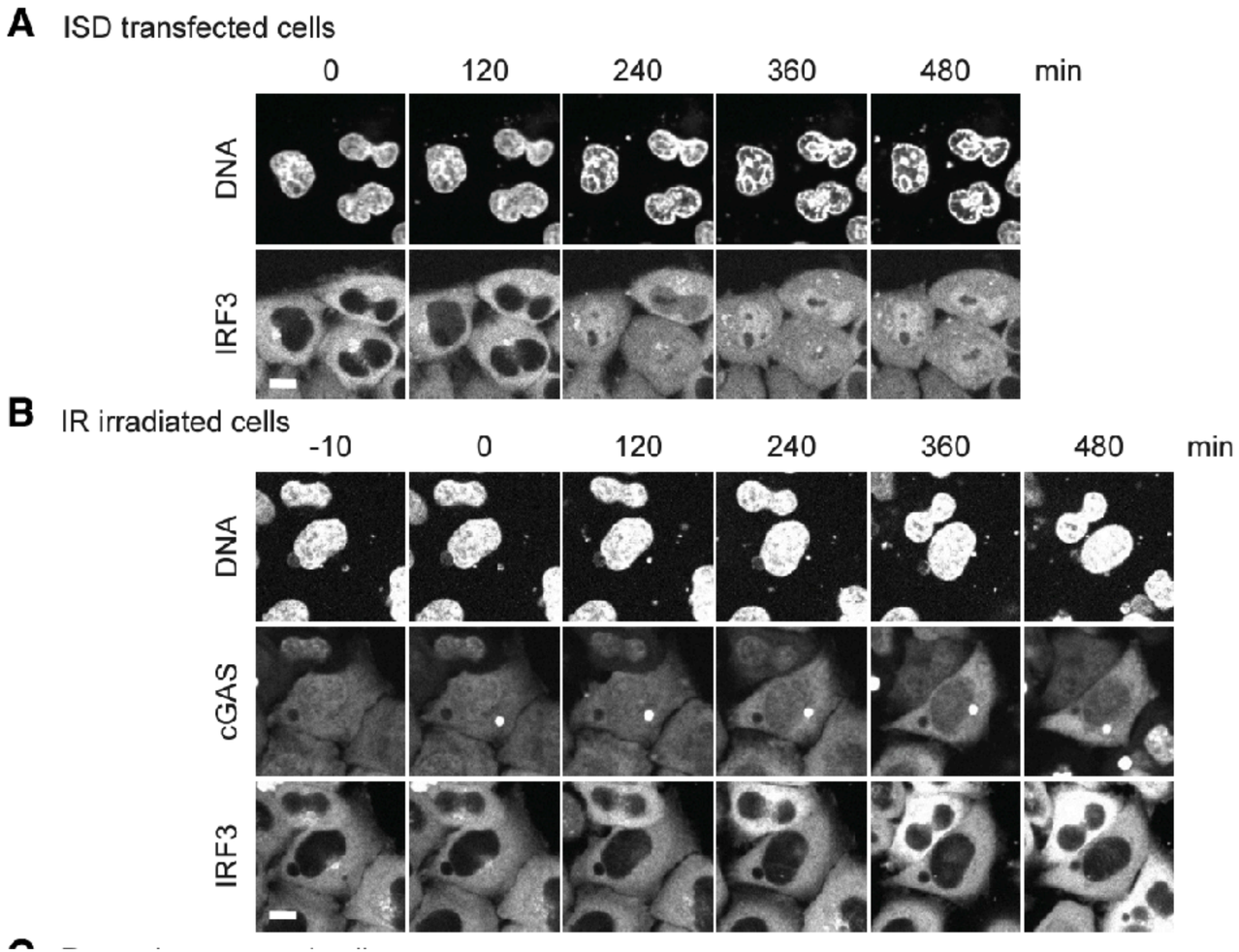
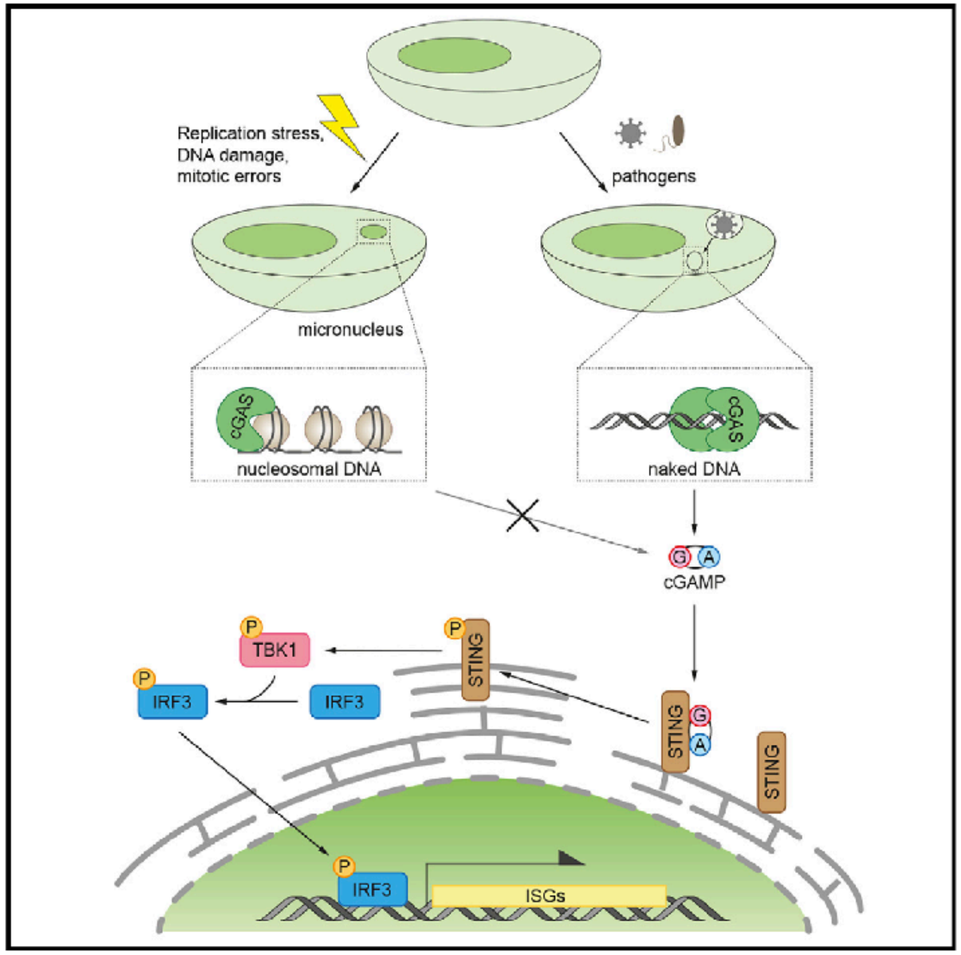


Do micronuclei really activate cGAS signaling?



Graphical abstract



Taki et al, *Mol Cell*, 2024.

Take home points

- Centromeres ensure accurate chromosome segregation during mitosis & meiosis
- Centromeres serve as chromosomal attachment site to spindle
- Centromere sequences vary widely, but centromere proteins are largely conserved
- Centromeres are defined epigenetically via CENP-A deposition
- Kinetochore assembles over centromere during mitosis
- Aneuploidy & chromosomal instability are common in cancer
- CIN generates micronuclei
- Micronuclei exhibit DNA damage
- Micronuclei activate cGAS-STING signaling