Molecular evolution of the process of chromosome segregation: Lessons from fungal pathogens

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JNCASR, Bangalore

Indian Academy of Sciences
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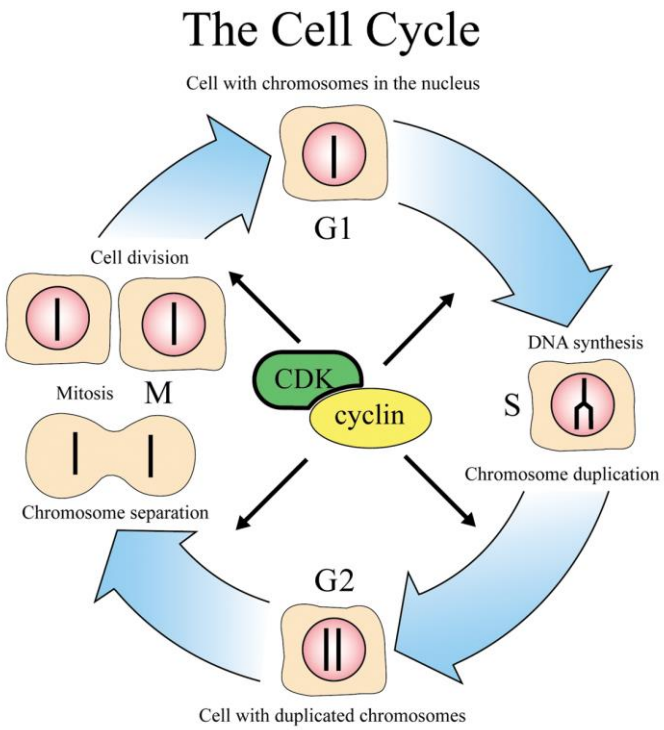
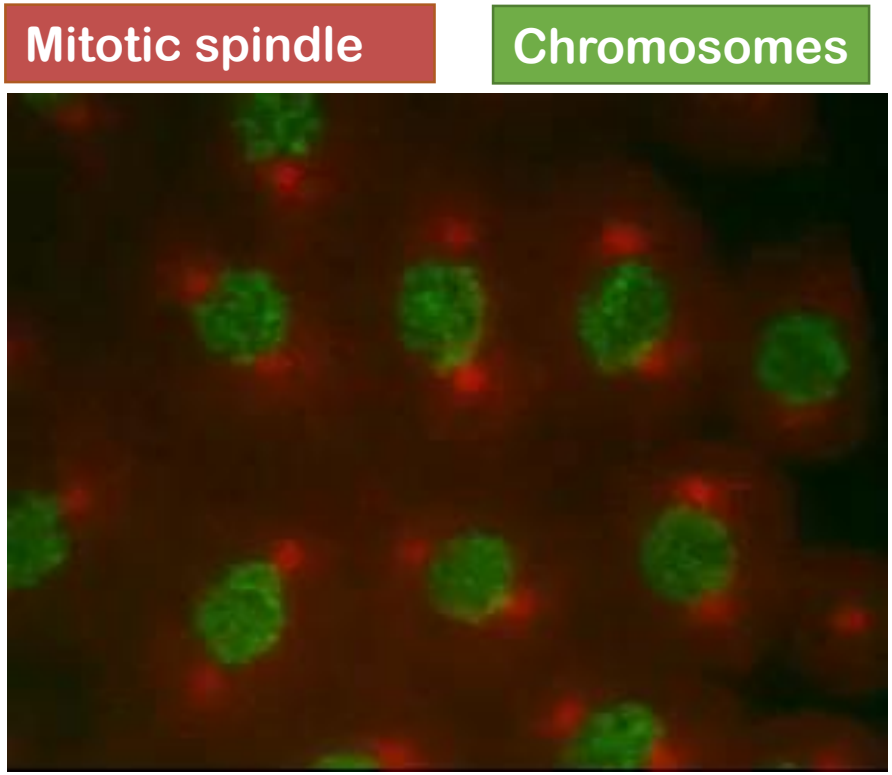
03 – 05 November 2017

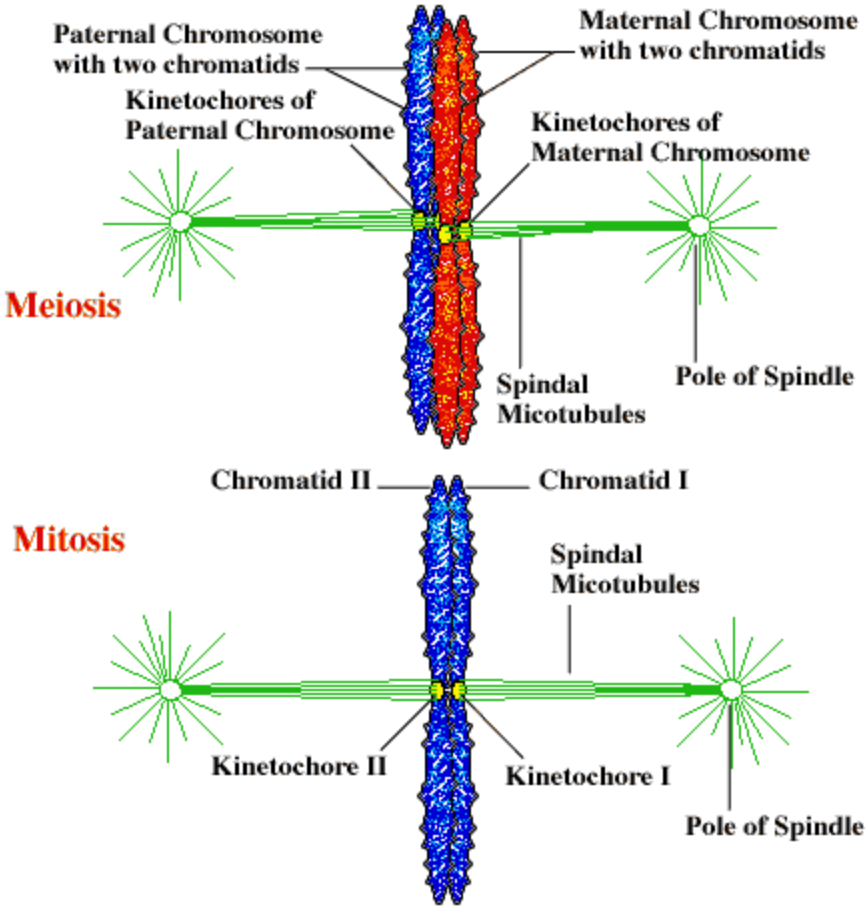
The process of chromosome segregation





http://nobelprize.org/medicine/laureates/2001/press.html

The centromere-kinetochore complex



http://ntri.tamuk.edu/cell/an-kinetochore.gif

CENTROMERE

***Cis*-acting DNA locus**

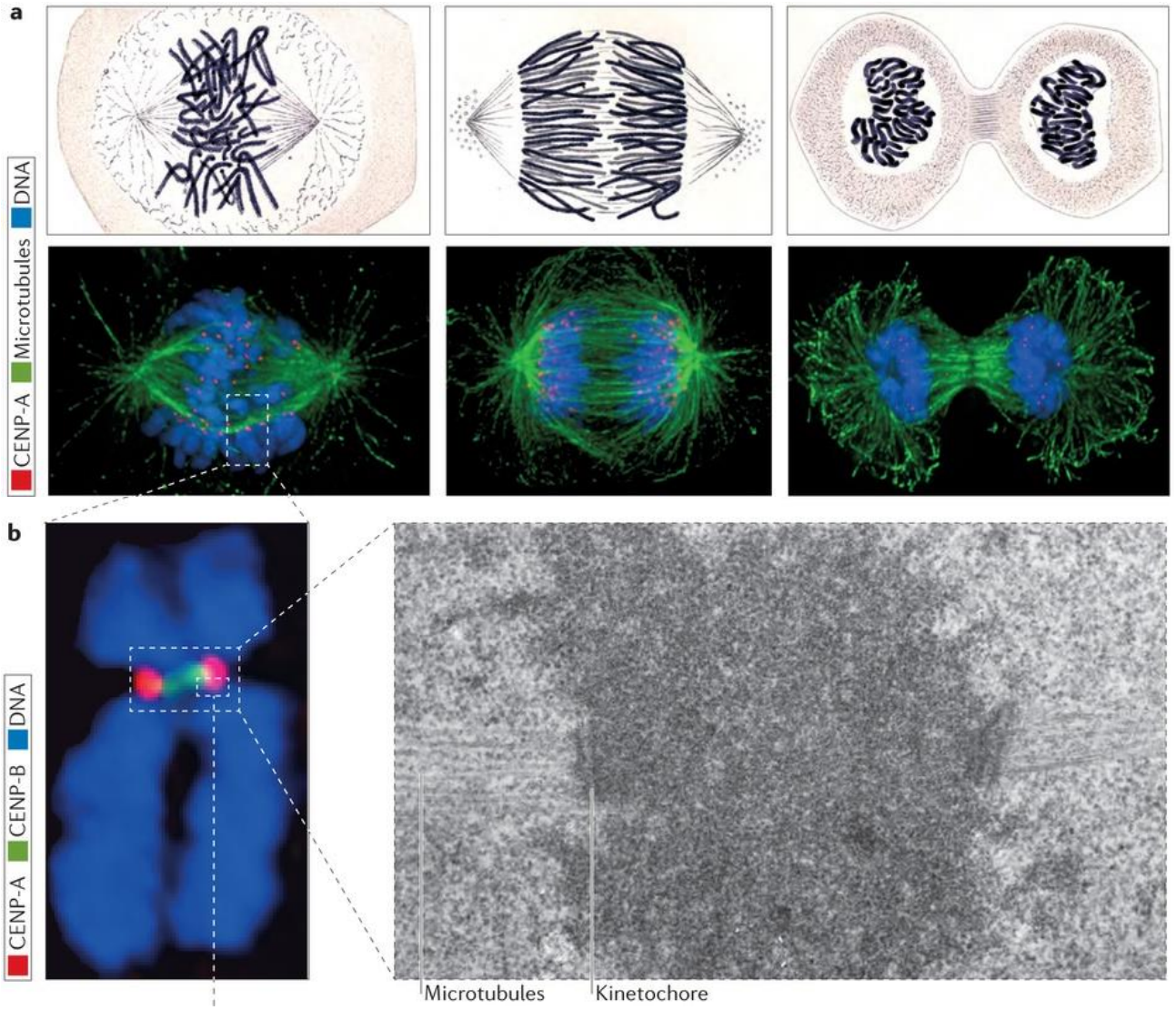
Site of protein recruitment, microtubule attachment and sister chromatid cohesion

thus it controls faithful chromosome segregation during mitosis and meiosis

KINETOCHORE

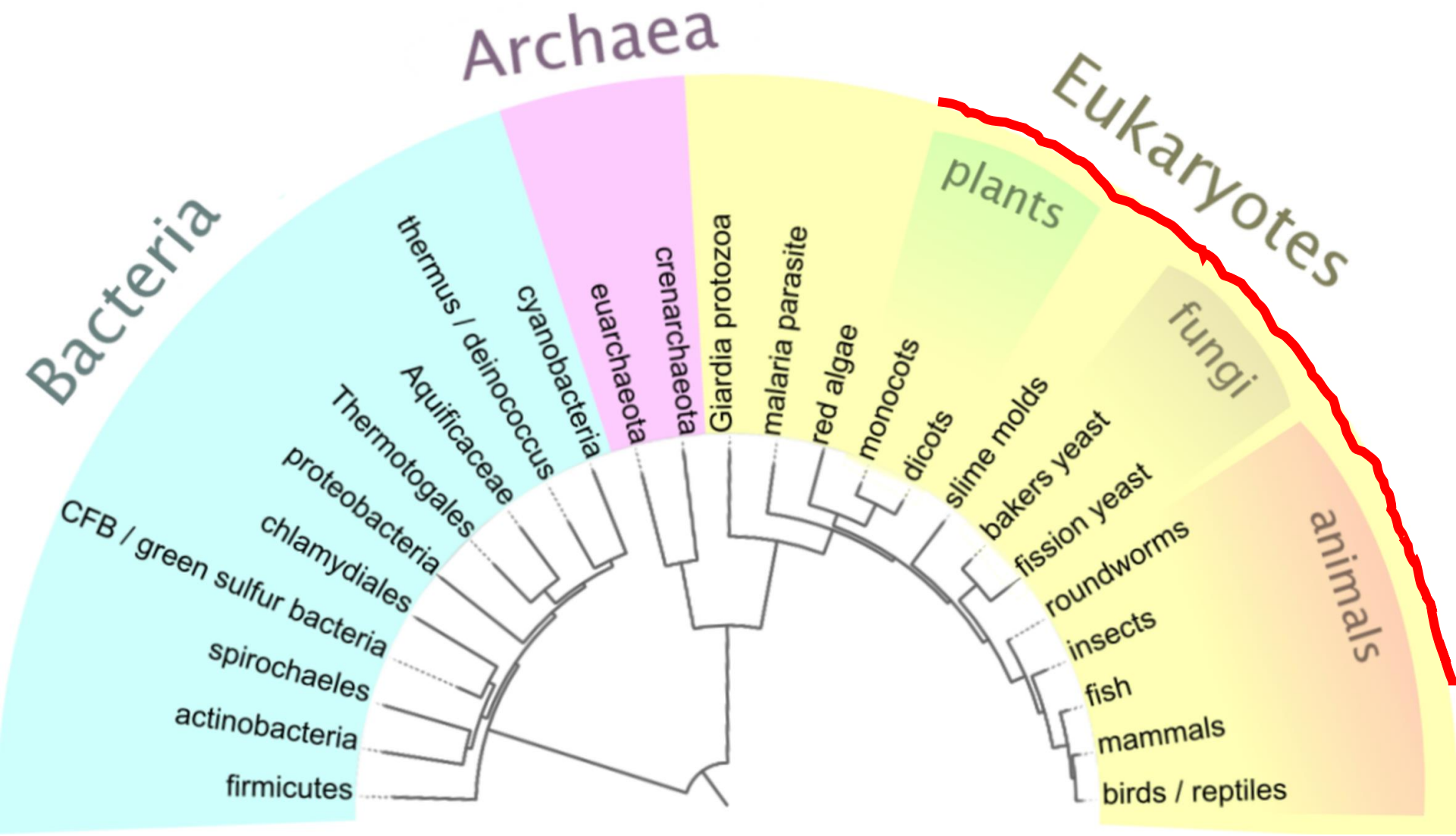
Proteinaceous structure made up of *trans*-acting factors that interface between the centromere and the spindle microtubules.

The centromere-kinetochore complex

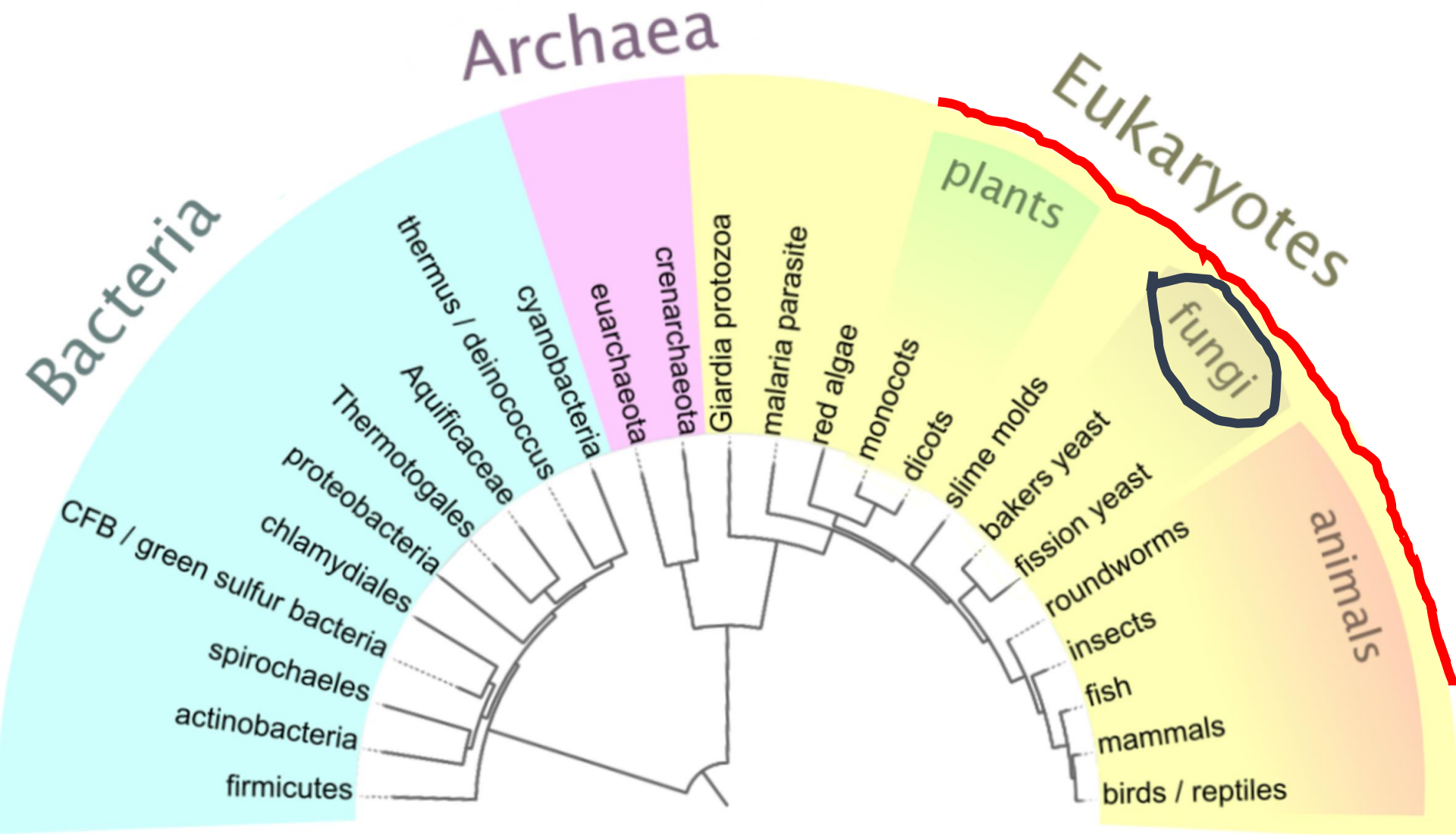


McKinley and Cheeseman, 2016, Nat Rev Mol Cell Biol

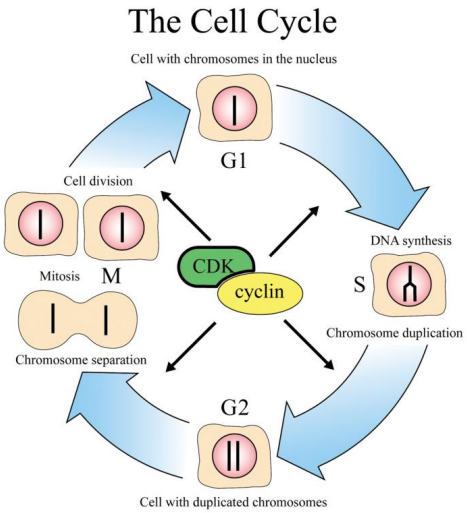
The tree of life

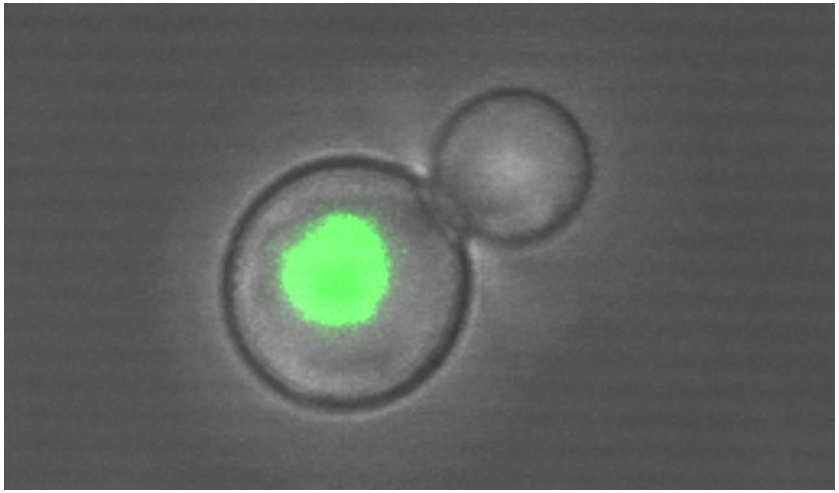


The tree of life



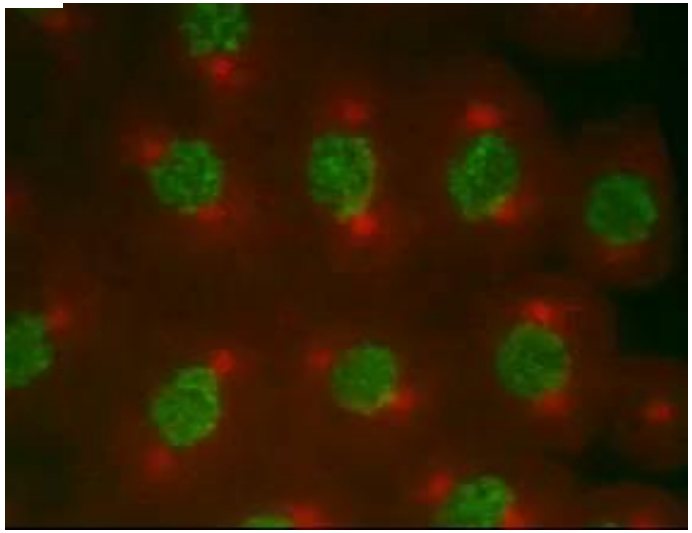
The process of chromosome segregation





Histone H4-GFP

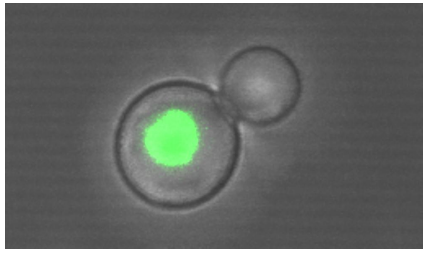
No metaphase plate



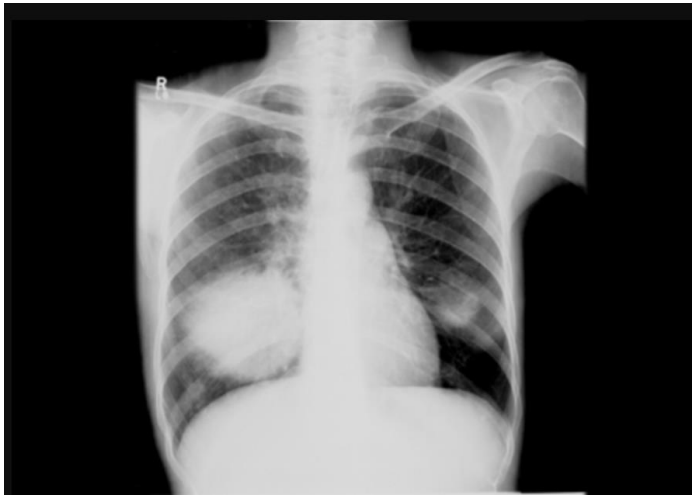
Mitotic spindle

Chromosomes

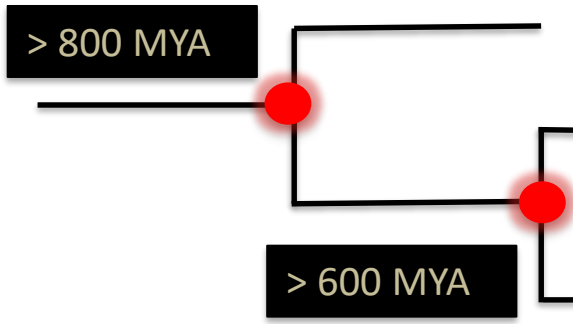
Evolution of centromeres in fungi



Cryptococcosis



Candidiasis



Cryptococcus neoformans

Candida albicans

Saccharomyces cerevisiae

human-fruit fly (~990 MYA)

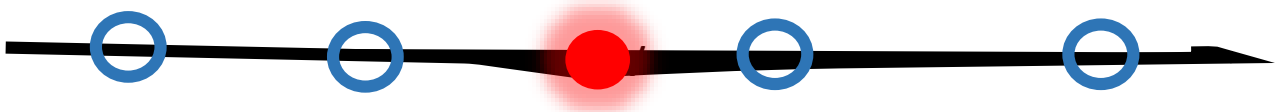
human-zebrafish (~450 MYA)

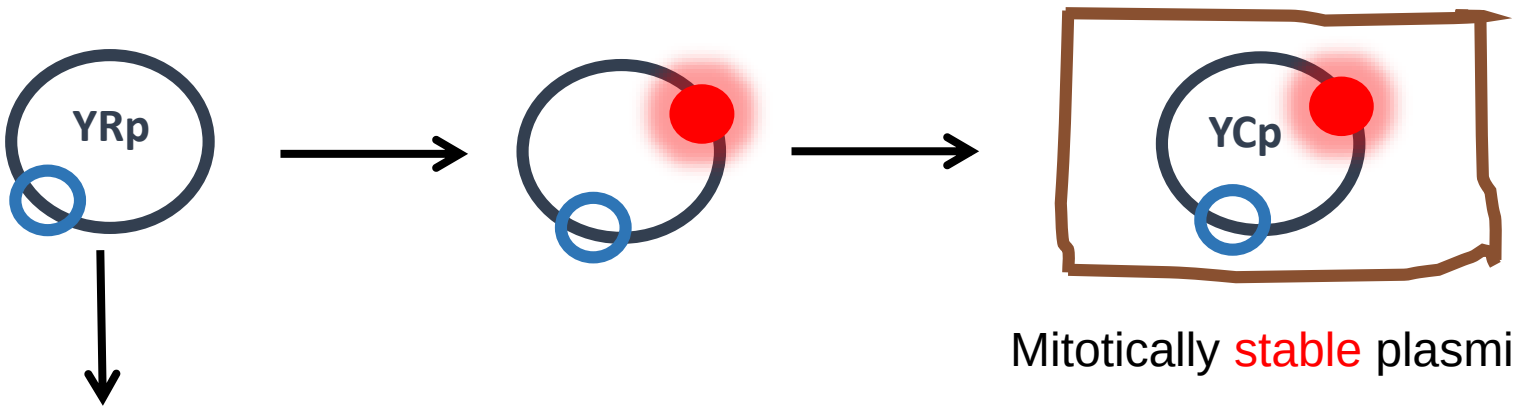
A centromeric plasmid is stably propagated in yeast

CEN

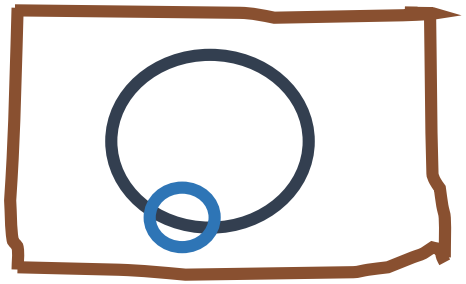
Origin

Saccharomyces





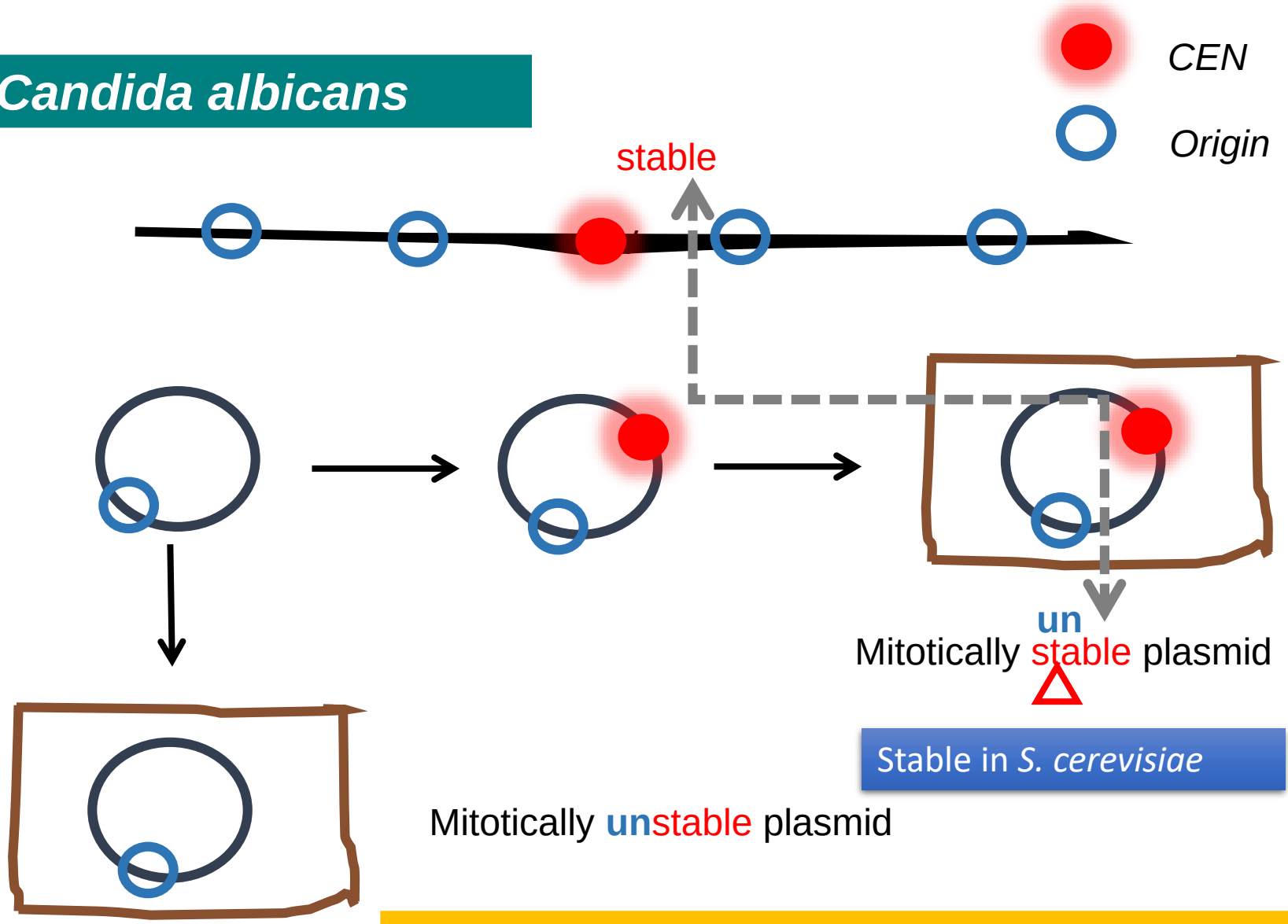
Mitotically stable plasmid



Mitotically unstable plasmid

Conundrum of centromere biology

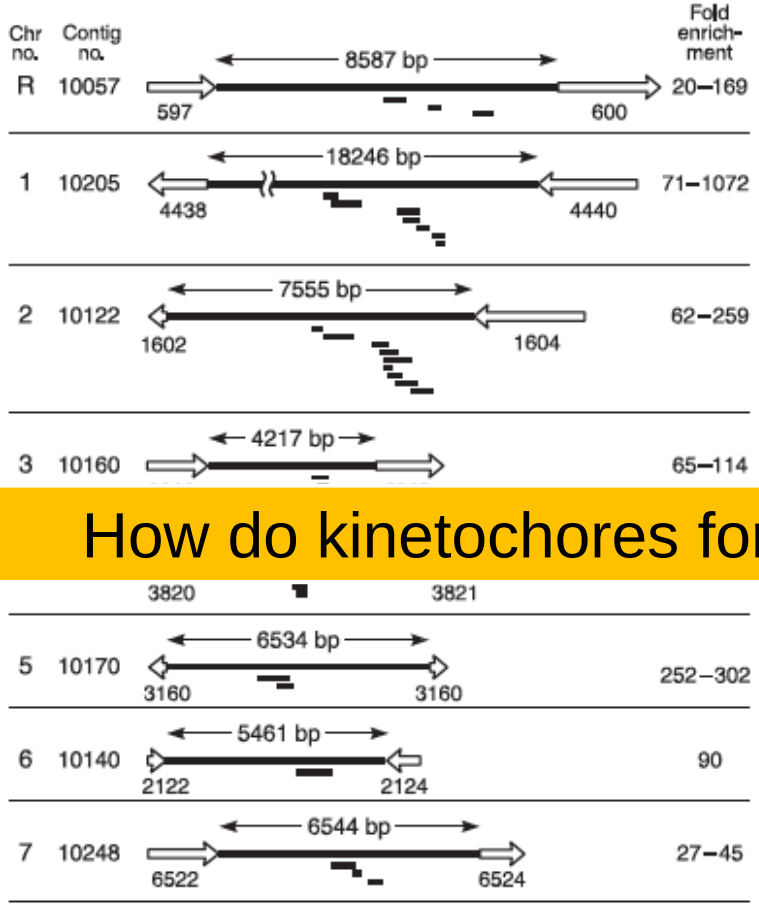
Candida albicans



DNA sequence cues are insufficient for CEN identity

Candida albicans centromeres

No common sequence motifs or repeats



Candida albicans

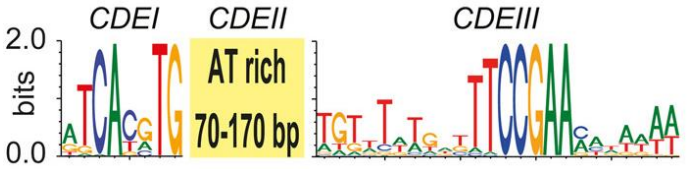
3-5 kb long centromeric chromatin

No common sequence motifs

How do kinetochores form on different DNA sequences?

Saccharomyces cerevisiae

Conventional point centromere

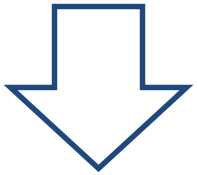


Kobayashi et al., 2015, Current Biology

Centromere identity crisis

Each CEN in *Candida albicans* is unique and different

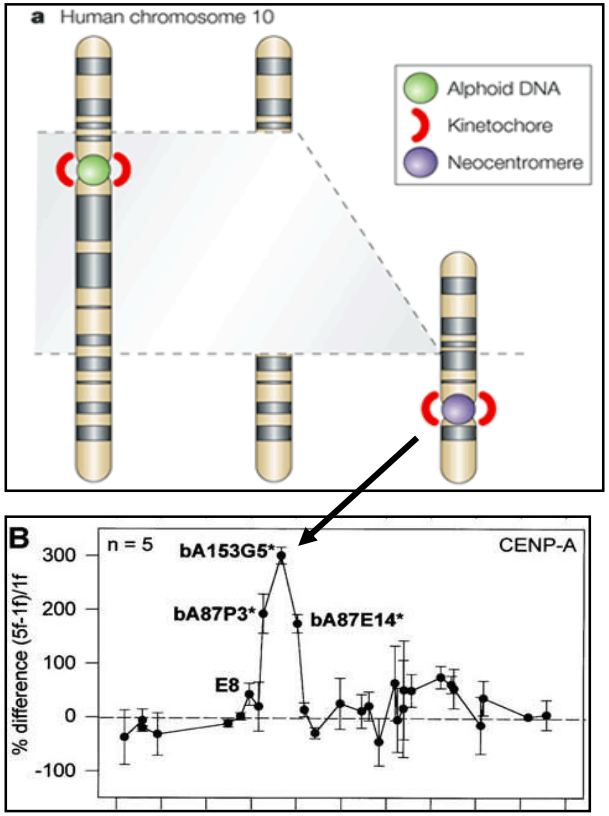
Genomic context but not the DNA sequence confers CEN function



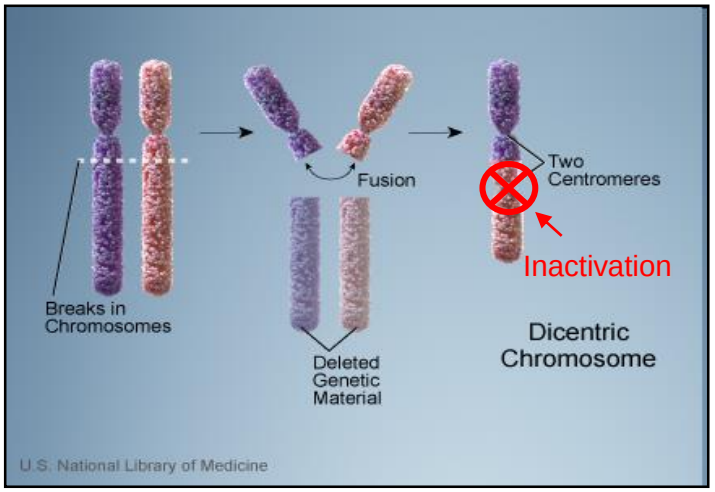
How does CENP-A know where to go?

Non-DNA sequence determinants?

DNA sequence cues are insufficient for CEN identity

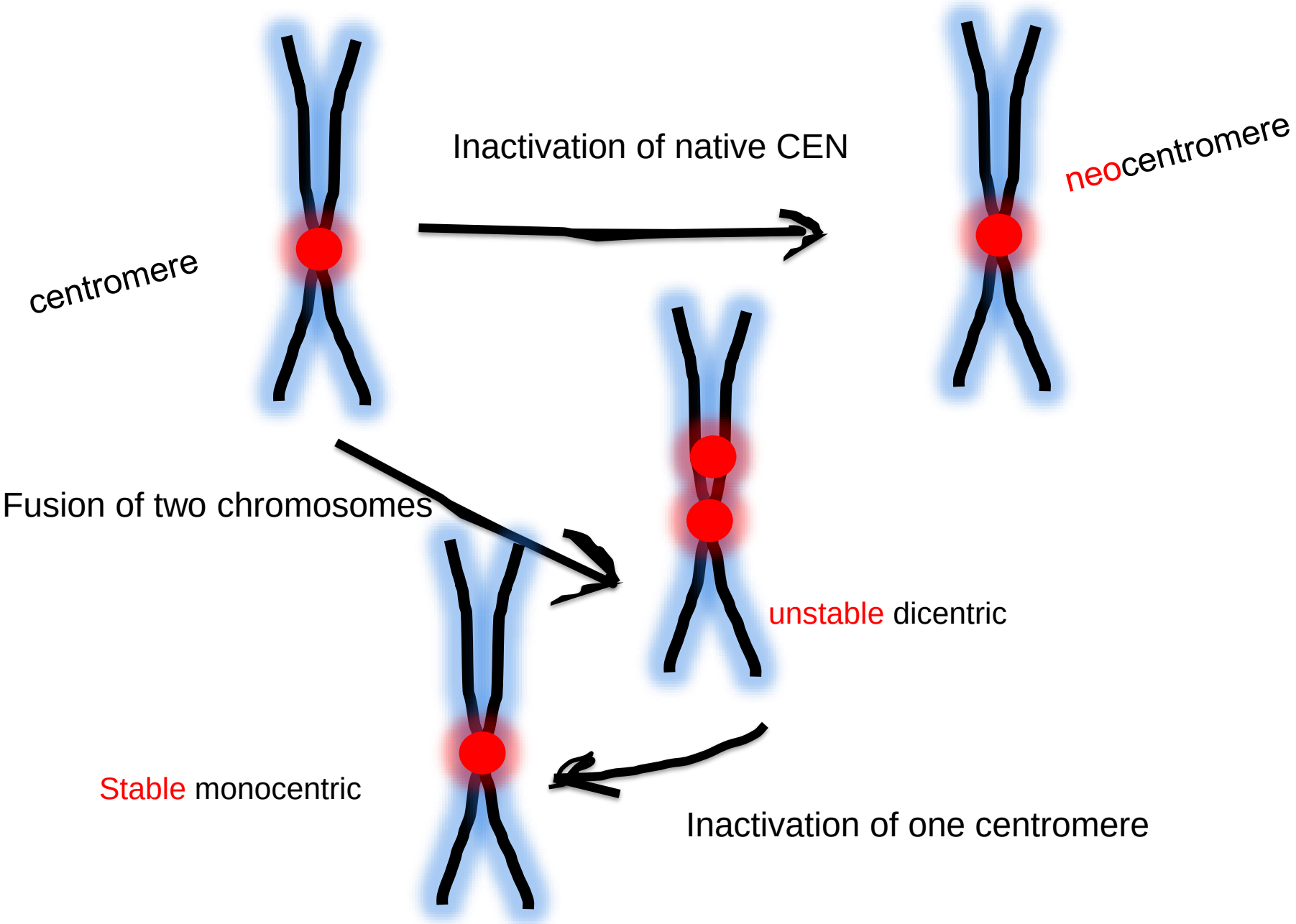


Neocentromeres

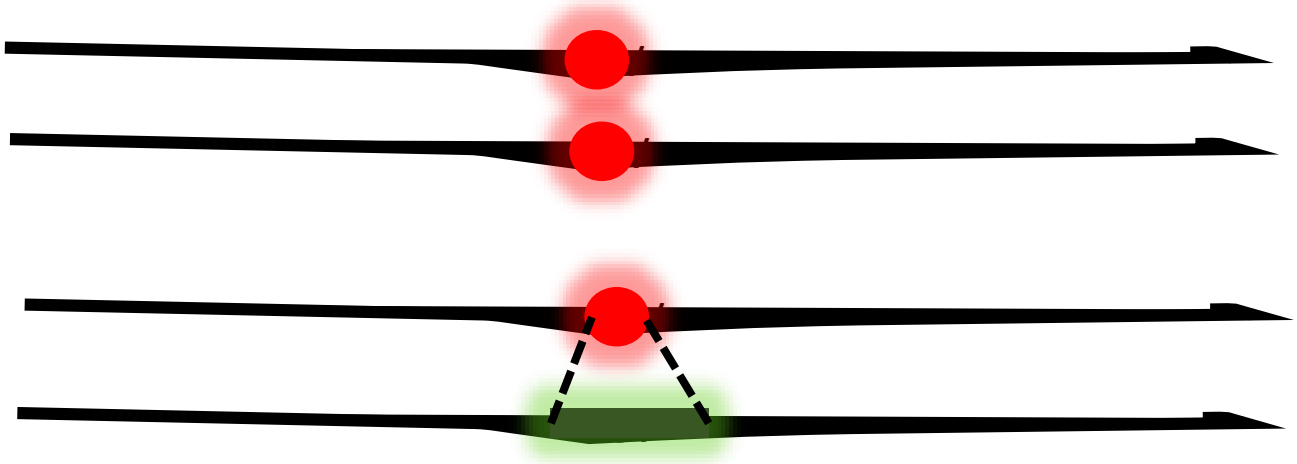


Inactivation of CEN in a dicentric chromosome

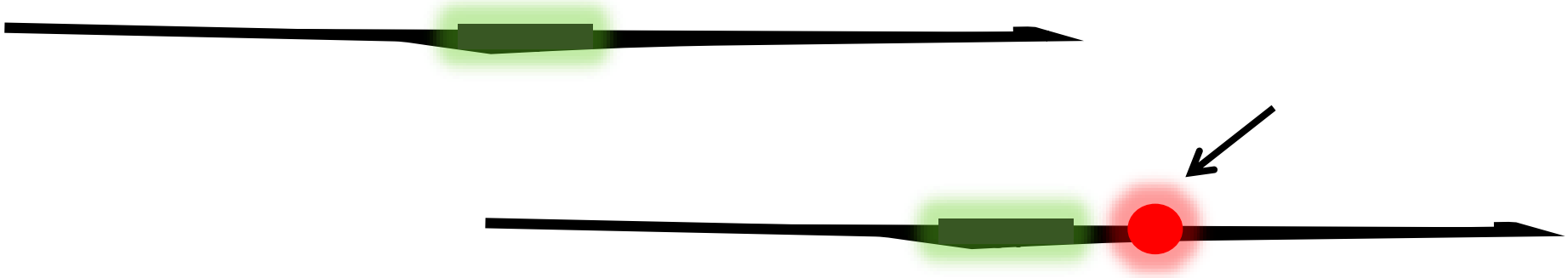
DNA sequence cues are insufficient for CEN identity



Fate of a chromosome when the native CEN is deleted

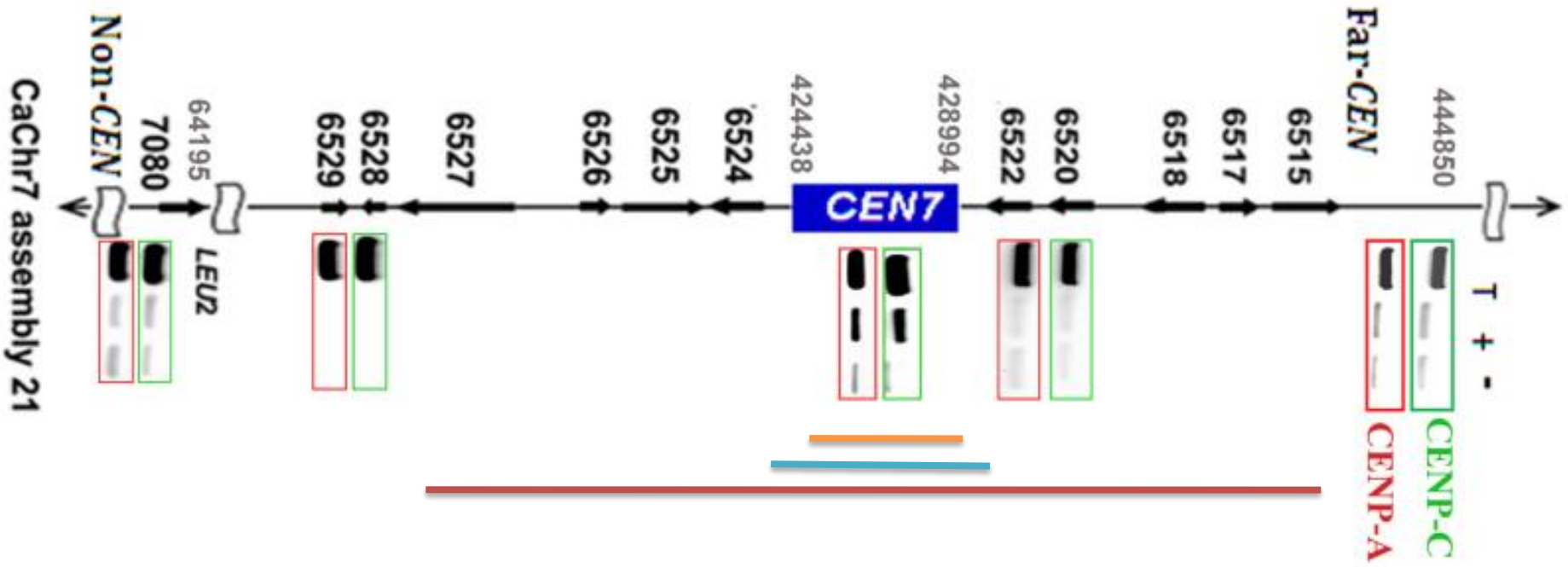


No centromere > unstable chromosome > chromosome loss **(2n-1)**



Neocentromere > stable chromosome > no chromosome loss **(2n)**

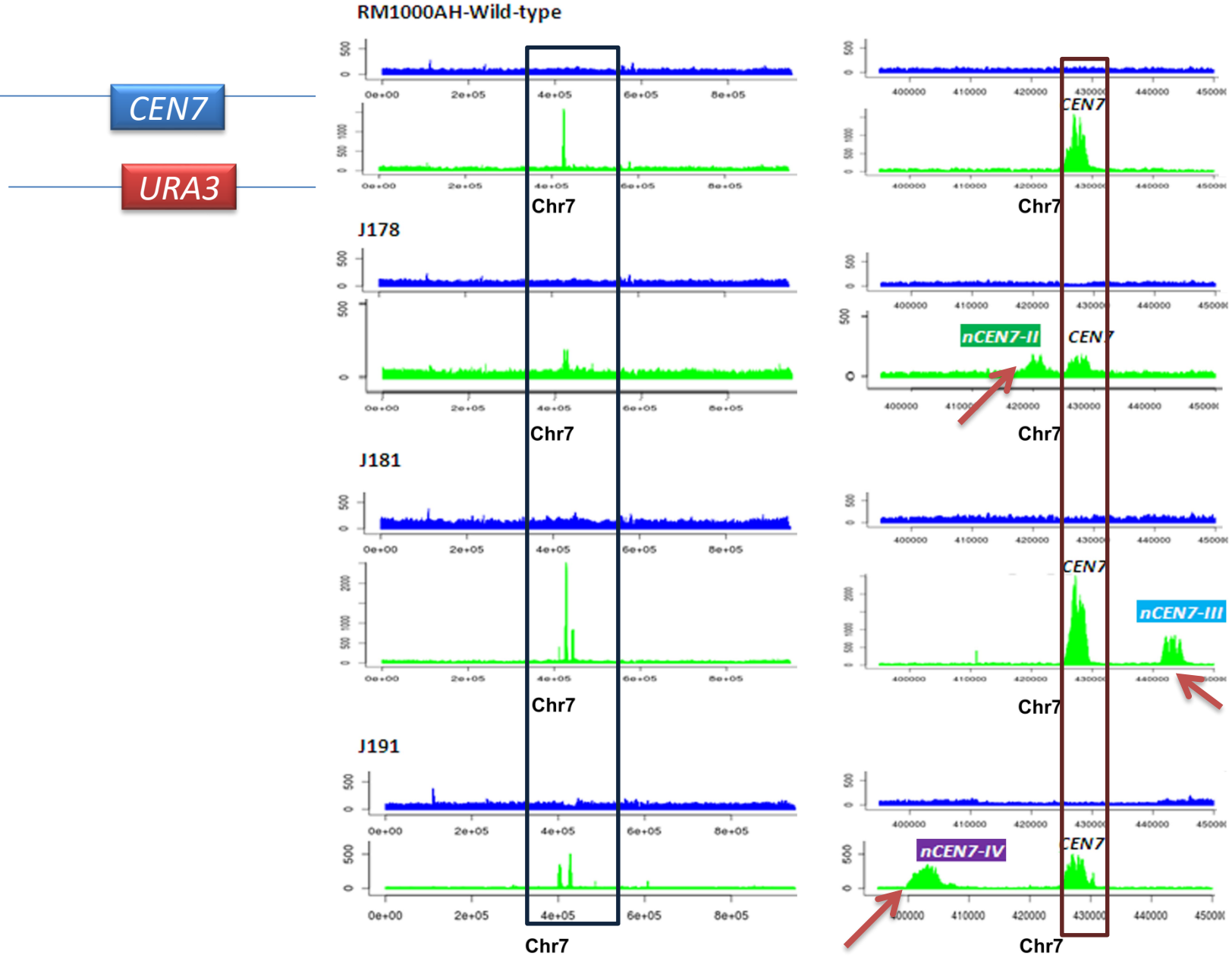
Dynamics of neocentromere formation in *C. albicans*



1. **Delete CENP-A rich region** **:4.5 kb**
2. **Delete ORF-free region** **:6.5 kb**
3. **Delete a long region** **: 30 kb**

Replace by 1.4 kb *URA3*

ChIP-sequencing confirms neocentromere locations are within 15 kb from the CEN

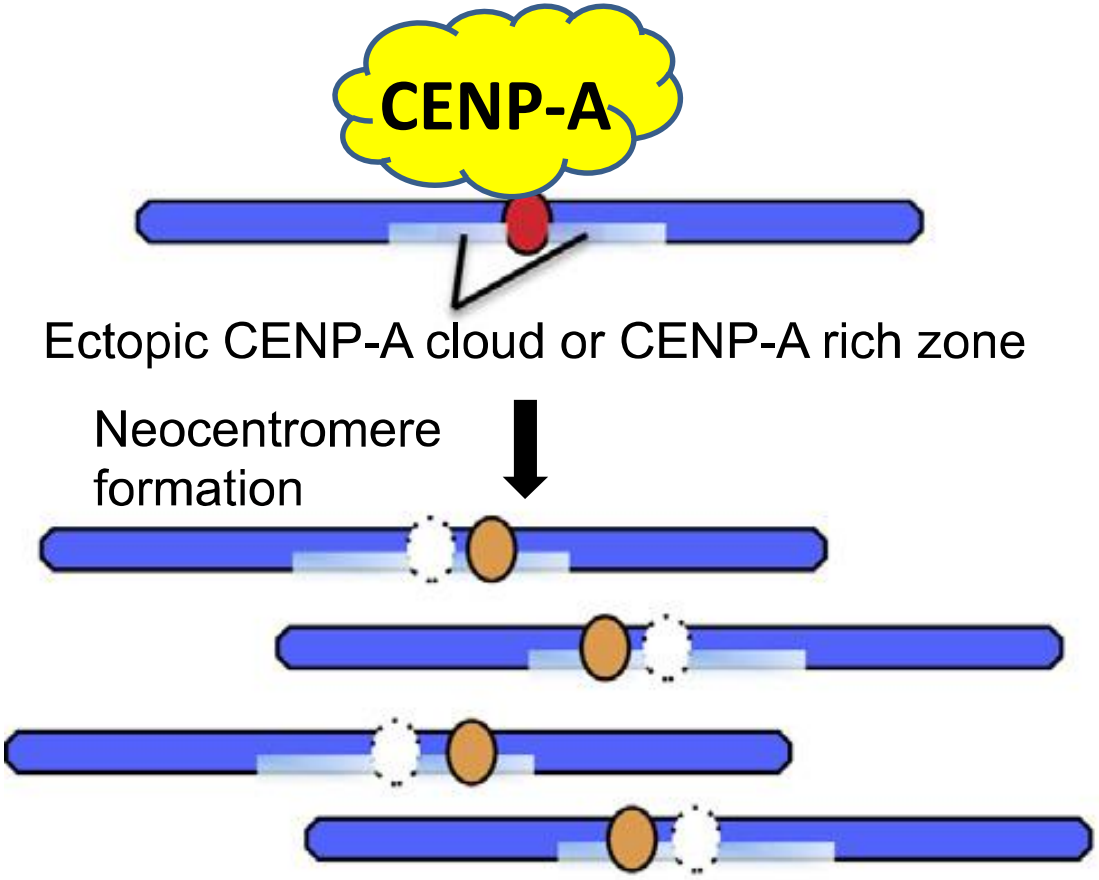


Epigenetic determinant

Chromosome conformation and CENP-A cloud

Thakur & Sanyal (2013) Genome Research

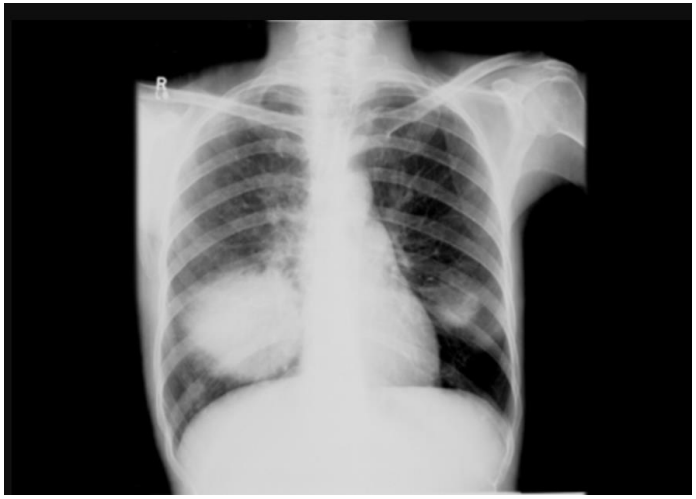
Neocentromeres in various organisms



Fukagawa & Earnshaw (2014) Developmental Cell

Evolution of centromeres in fungi

Cryptococcosis



Candidiasis

> 800 MYA

Cryptococcus neoformans

?

Candida albicans

DNA sequence independent

3000 bp

> 600 MYA

Saccharomyces cerevisiae

DNA sequence

125 bp

human-fruit fly (~990 MYA)

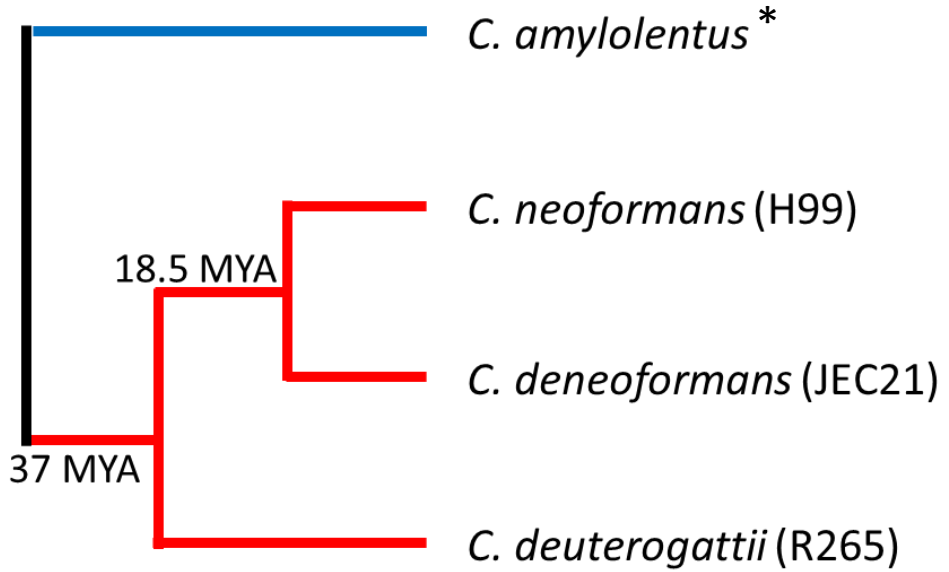
human-zebrafish (~450 MYA)

Centromeres in closely related *Cryptococcus* species?

Cryptococcus

**Sheng/Yadav et al., (2017) PLOS Biology*

Vikas Yadav



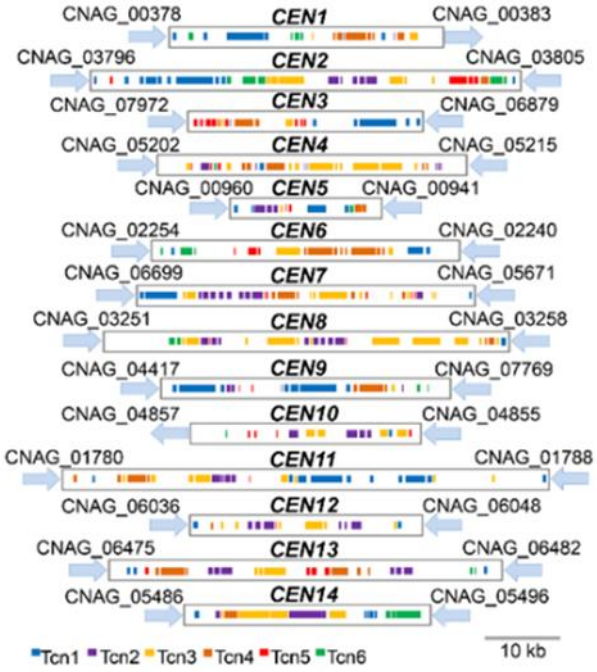
In collaboration with Joe Heitman, Sheng Sun & Blake Billmyre (Duke University)

Centromeres are retrotransposon-rich in the *Cryptococcus* species complex

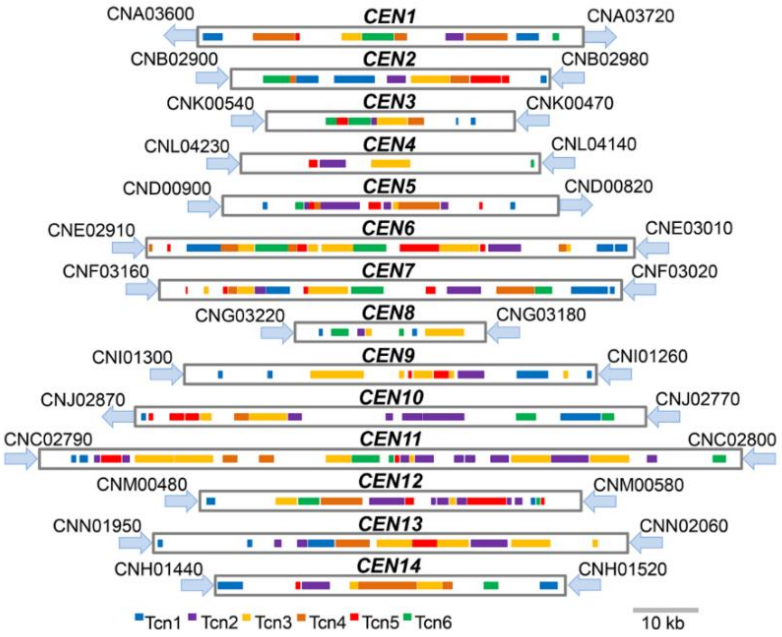
RNAi +

RNAi -

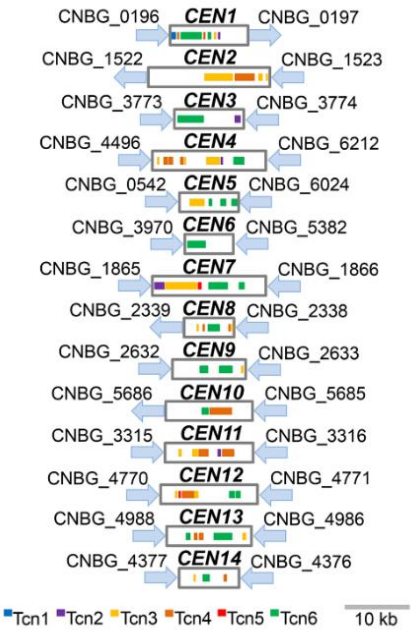
H99



JEC21



R265



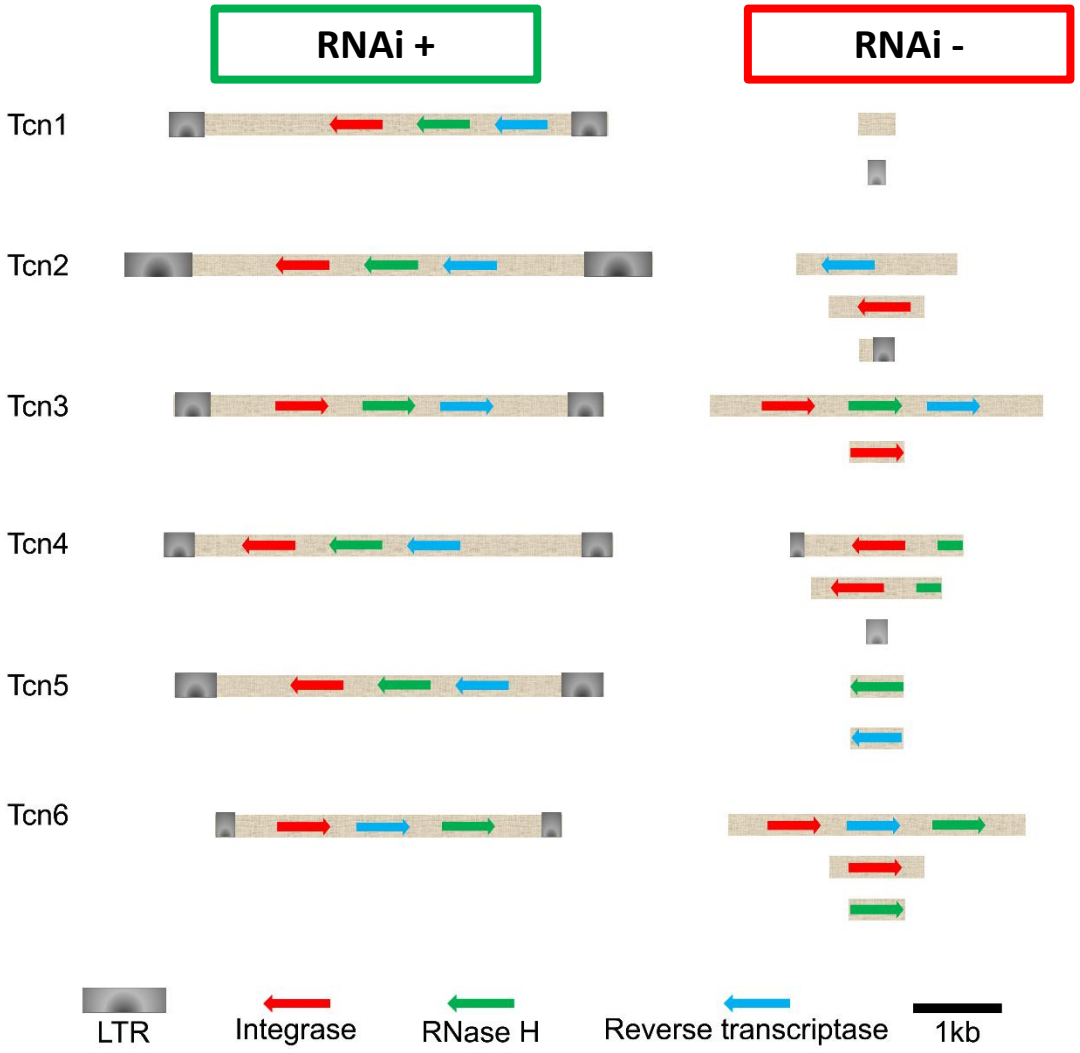
Identified by
CENP-A/CENP-C ChIPseq

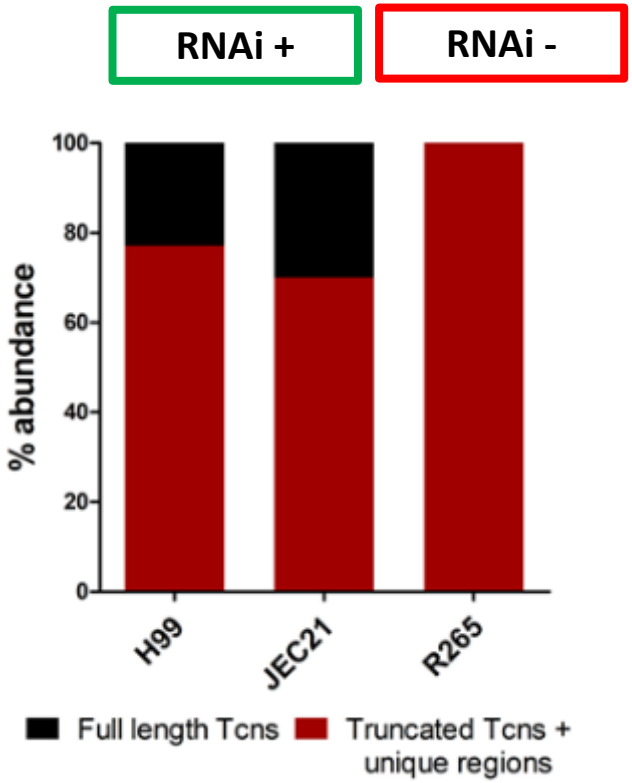
RNA interference
RNAi

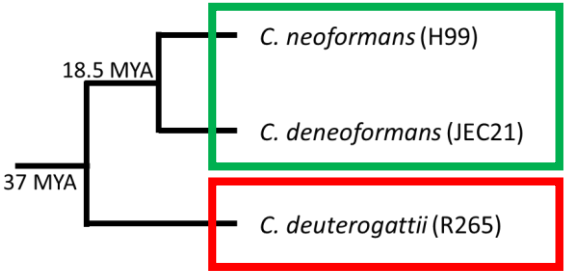
Argonaute
Dicer
RNA-dep RNA Pol

Silences genes

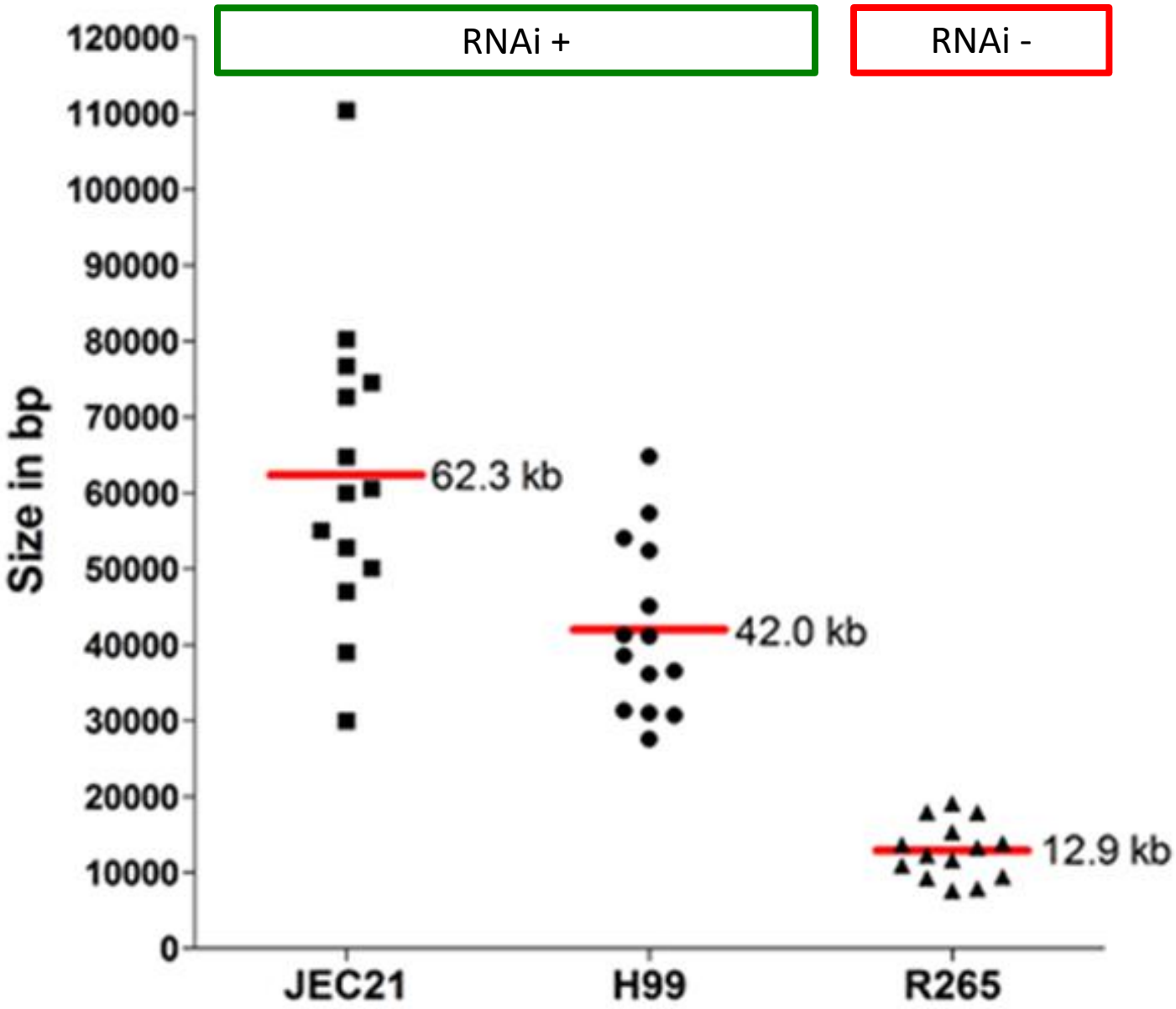
R265 centromeres harbour only truncated **retrotransposons**





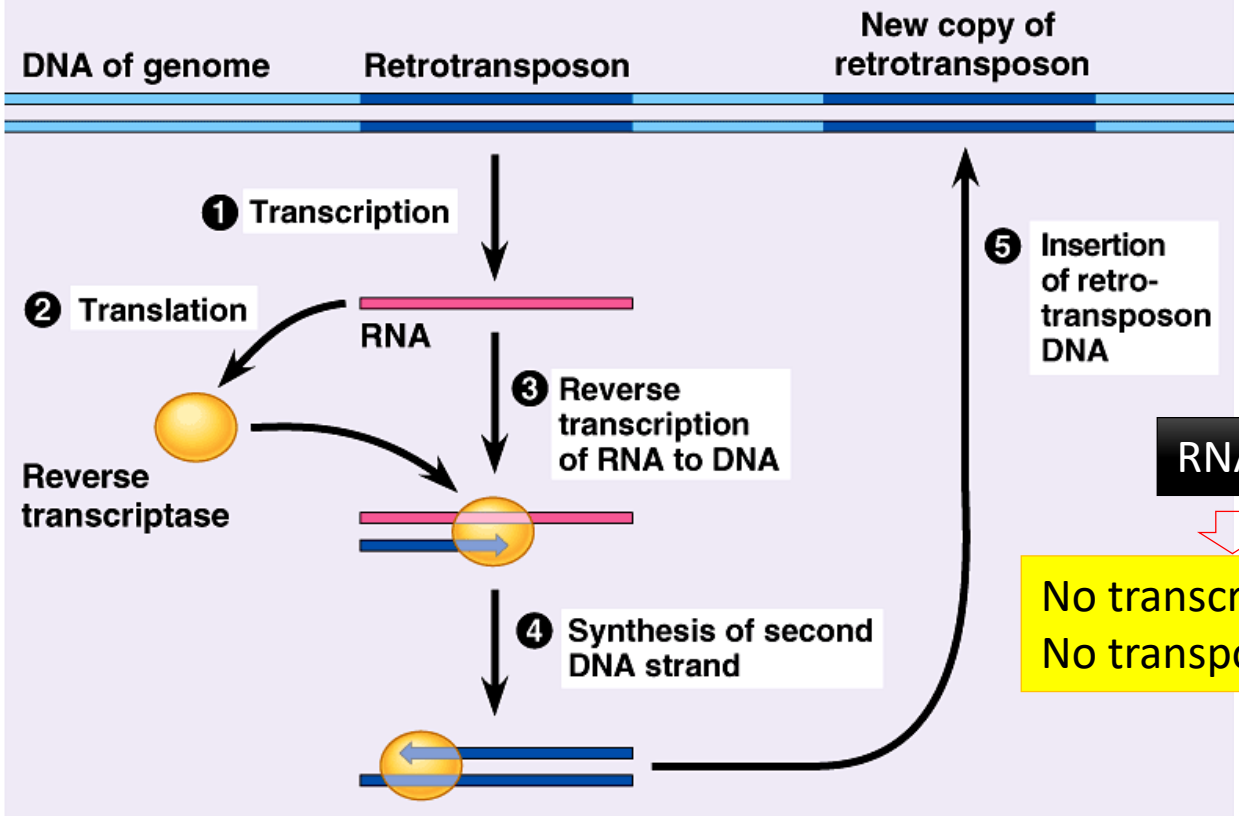


R265 (RNAi -) centromeres are smaller in size



RNA interference and centromeric heterchromatin maintenance

Transcription and amplification



RNAi and heterochromatin repress centromeric meiotic recombination

Chad Ellermeier[a,1], Emily C. Higuchi[a], Naina Phadnis[a], Laerke Holm[a,b], Jennifer L. Geelhood[a], Genevieve Thon[b], and Gerald R. Smith[a,2]

Janbon et al., 2010, Fungal Genet Biol.; Ellermeier et al., 2010, PNAS; Mirouze et al., 2012, PNAS; Devos et al., 2002, Genome Res; http://www.hammiverse.com/lectures/19/2.html.

RNAi-mediated centromere evolution - a proposed model

Loss of RNAi

Retrotransposons at the CEN

Increased transcription

Lack of RNAi

CEN region expansion

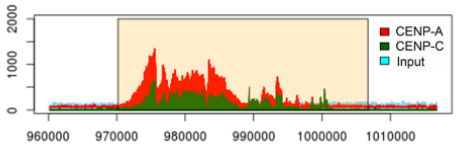
Increased recombination

Lack of DNAme

CEN region contraction

Transposon attrition followed by genome stablization

RNAi proficient strain
Large regional, transposon-rich centromeres (e.g. *C. neoformans*)

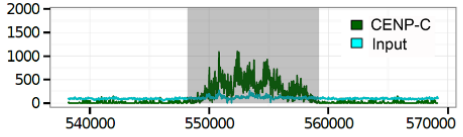


Loss of RNAi

Transposon attrition

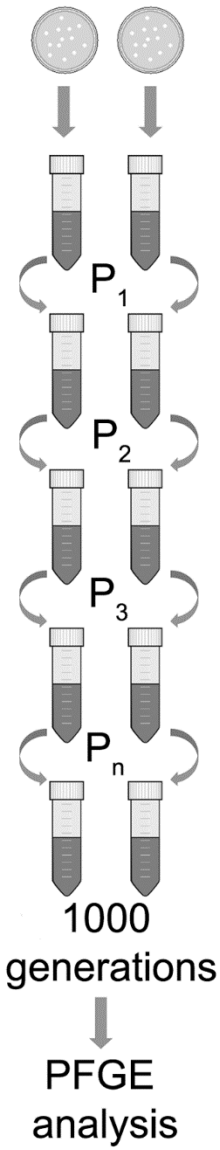
Reduction in length

RNAi deficient strain
Short regional, transposon-poor centromeres (e.g. *C. deuterogattii*)



Experimental evolution - Centromere length variation

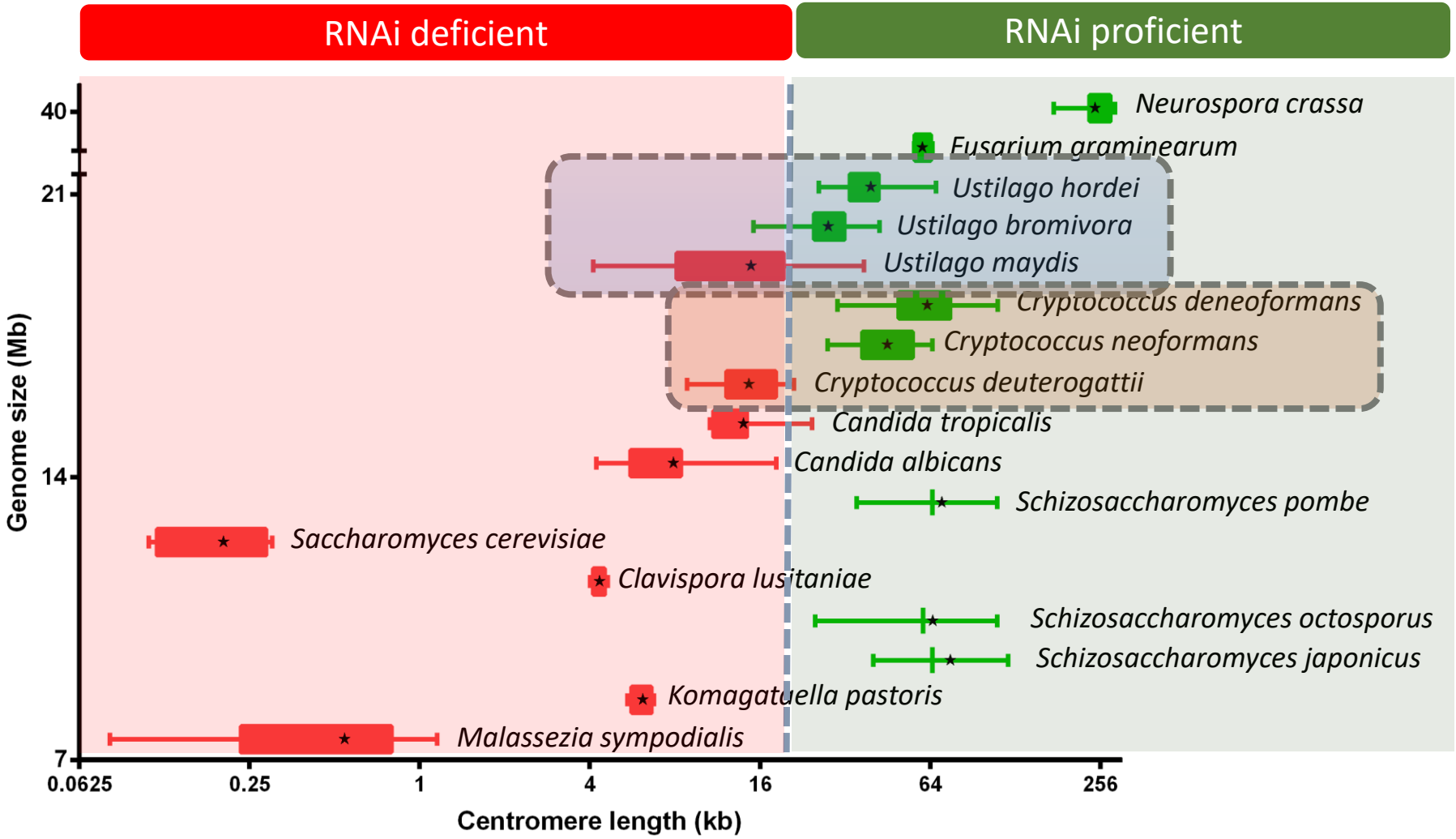
Plan: RNAi proficient strain > inactivate RNAi > grow 1000 generations



PacBio sequencing followed by de novo assembly of the genome

RNAi preserves the integrity of long centromeres

Vikas Yadav et al., PNAS (under revision)



The Molecular Mycology Laboratory

From **Left** to **right**: Krishnendu, Sundar, Kaustuv, Neha, Lakshmi, Radha, Priya, Vikas, Shreyas, Rima, Jigyasa, Bhavana and Laxmi Shanker

CSIR, DBT, SERB,
CEFIPRA (Indo-French), IJSPS (Indo-Japan)
JNCASR (intramural)
TATA innovation Fellowship

Rahul Siddharthan
DD Dubey
German Larriba
Joseph Heitman