Mechanisms of Chromosomal Fragility and Rearrangements Triggered by Human Unstable Repeats

Kirill Lobachev

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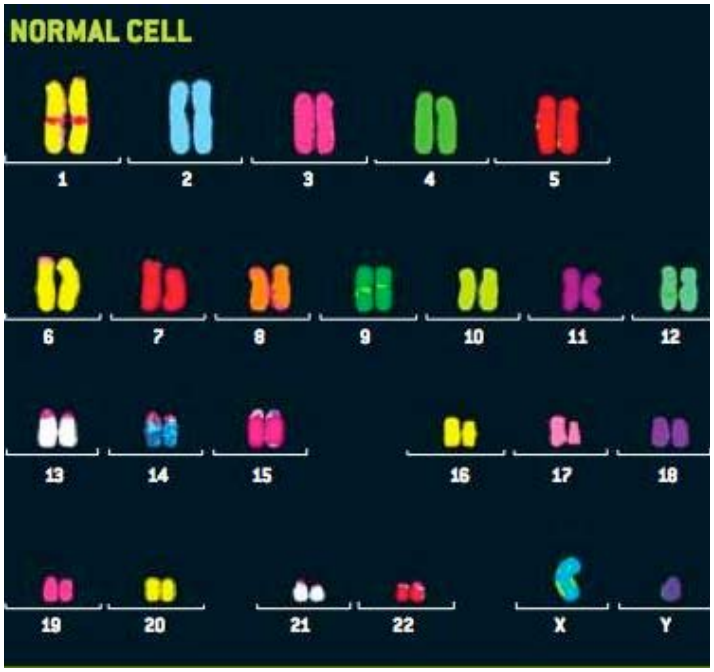
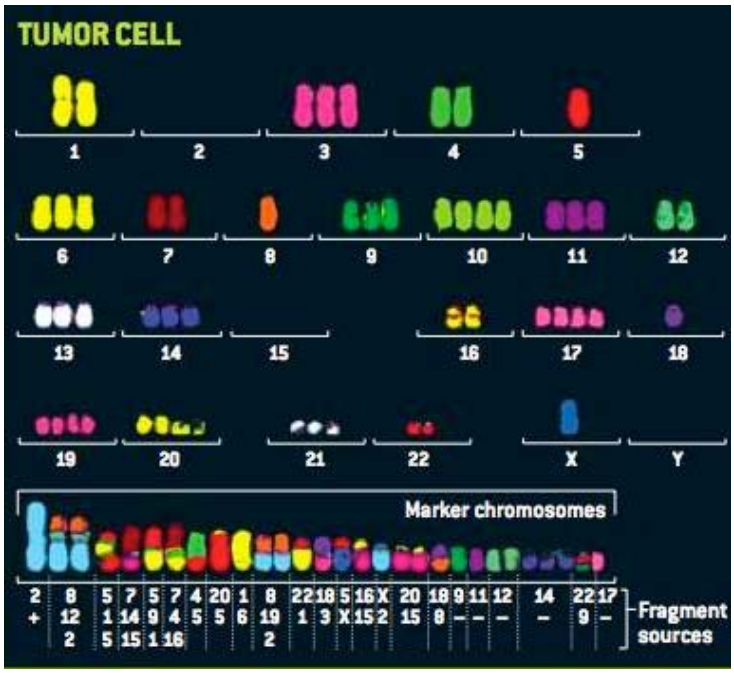
Atlanta

Majority of cancer cells are characterized by chromosome instability (CIN)





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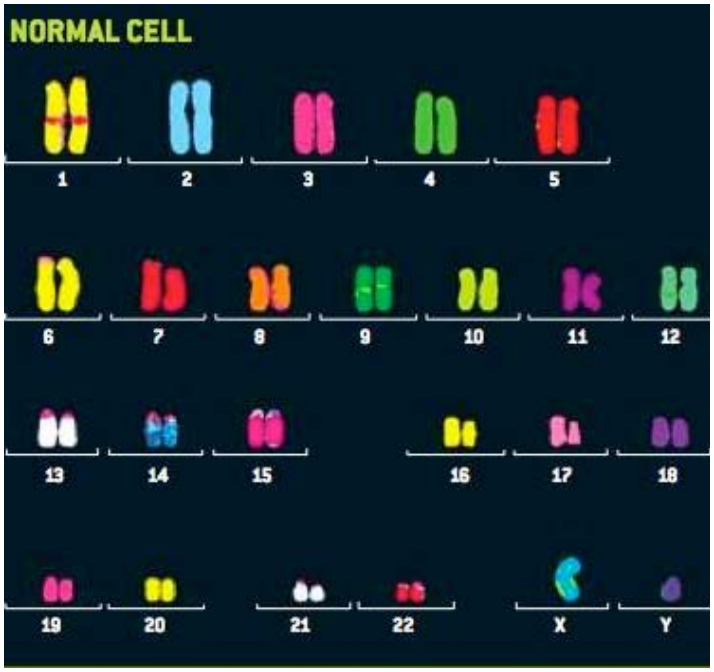
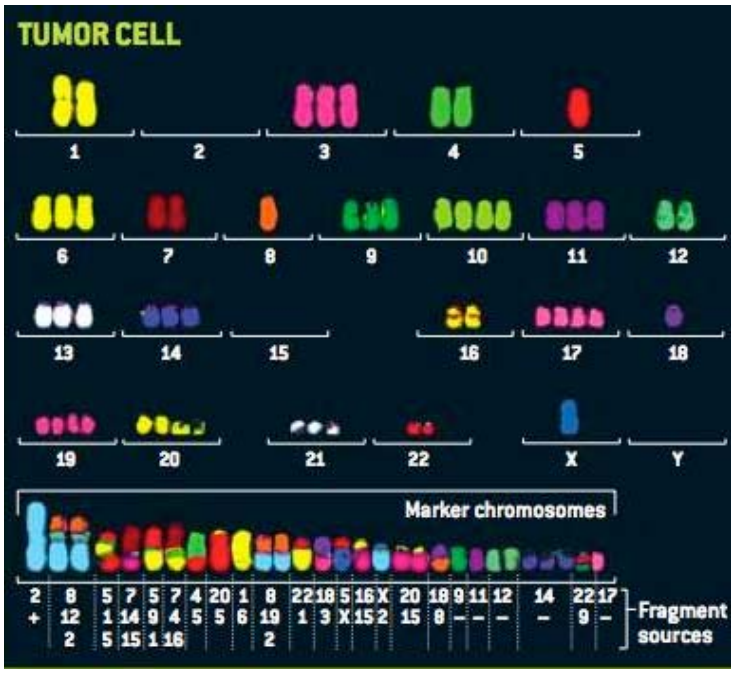
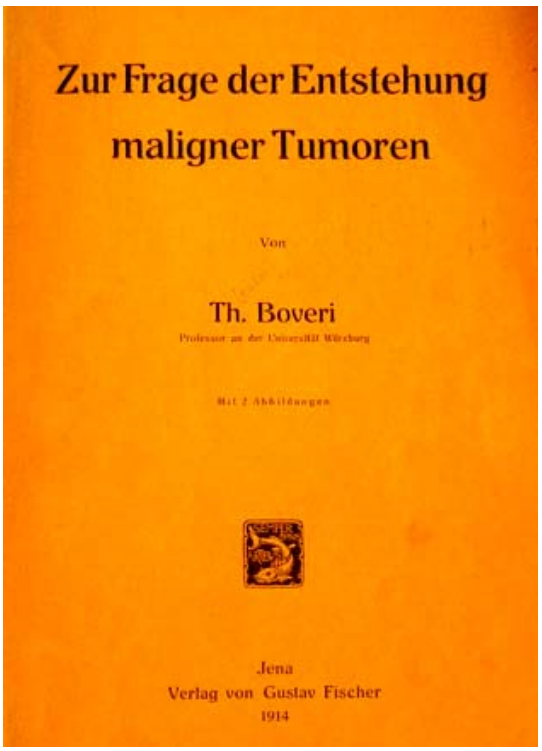


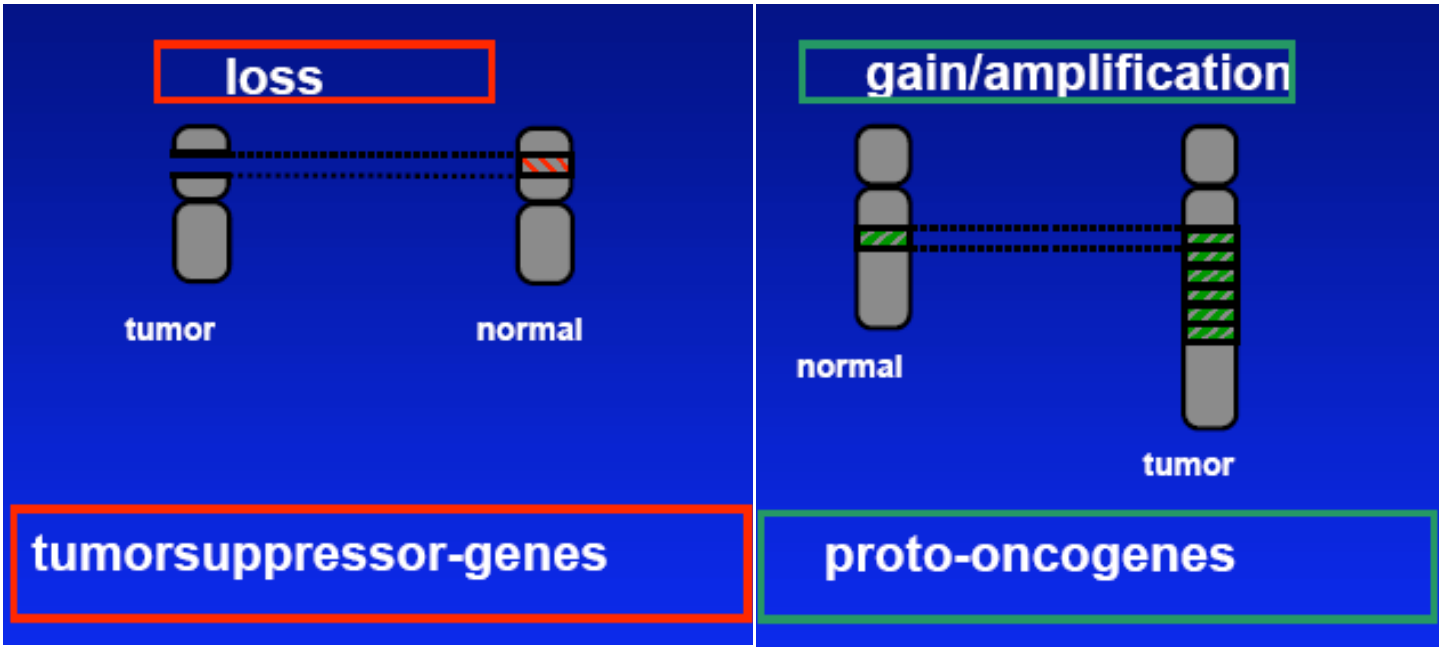


"Let me add…a consideration of the *inheritance of tumors*…In order that a tumor may arise in such cases, the homologous elements in both series of chromosomes must be weakened in the same way"

Theodor Boveri, pathologist, 1914



Carcinogenic chromosomal aberrations



impaired growth inhibition

increased proliferation

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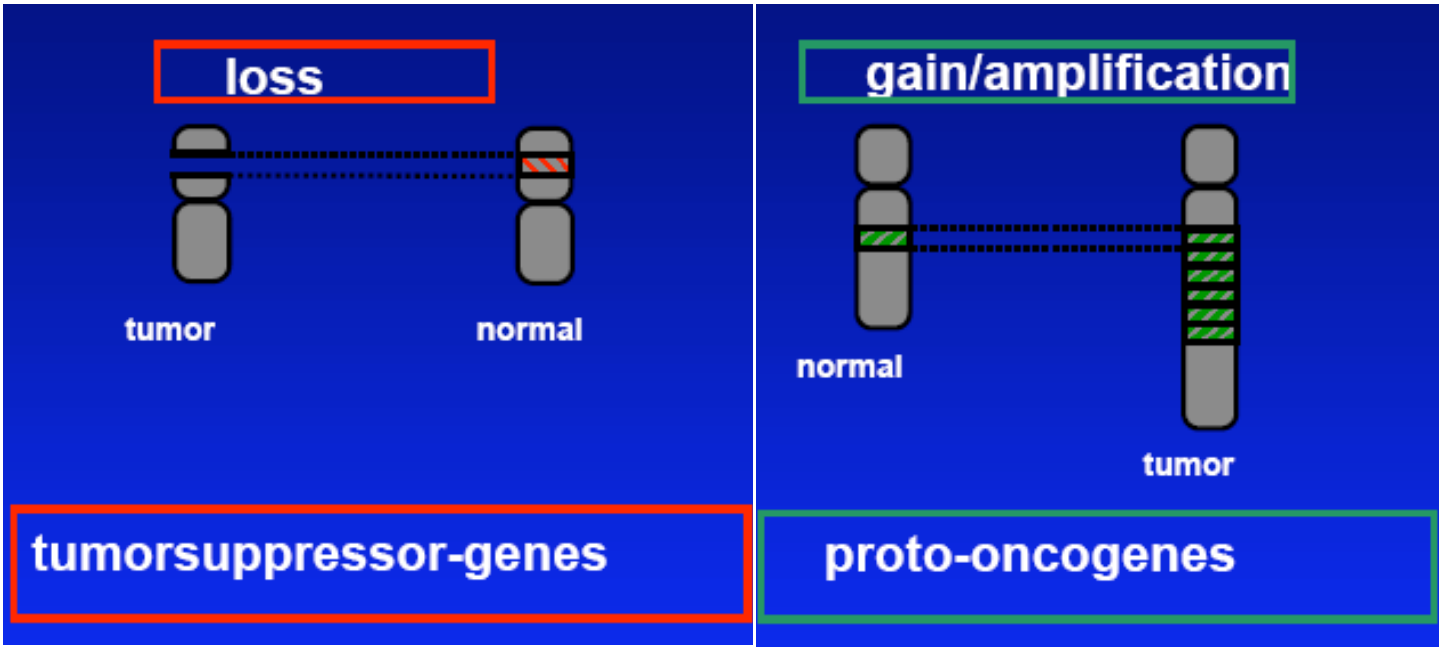

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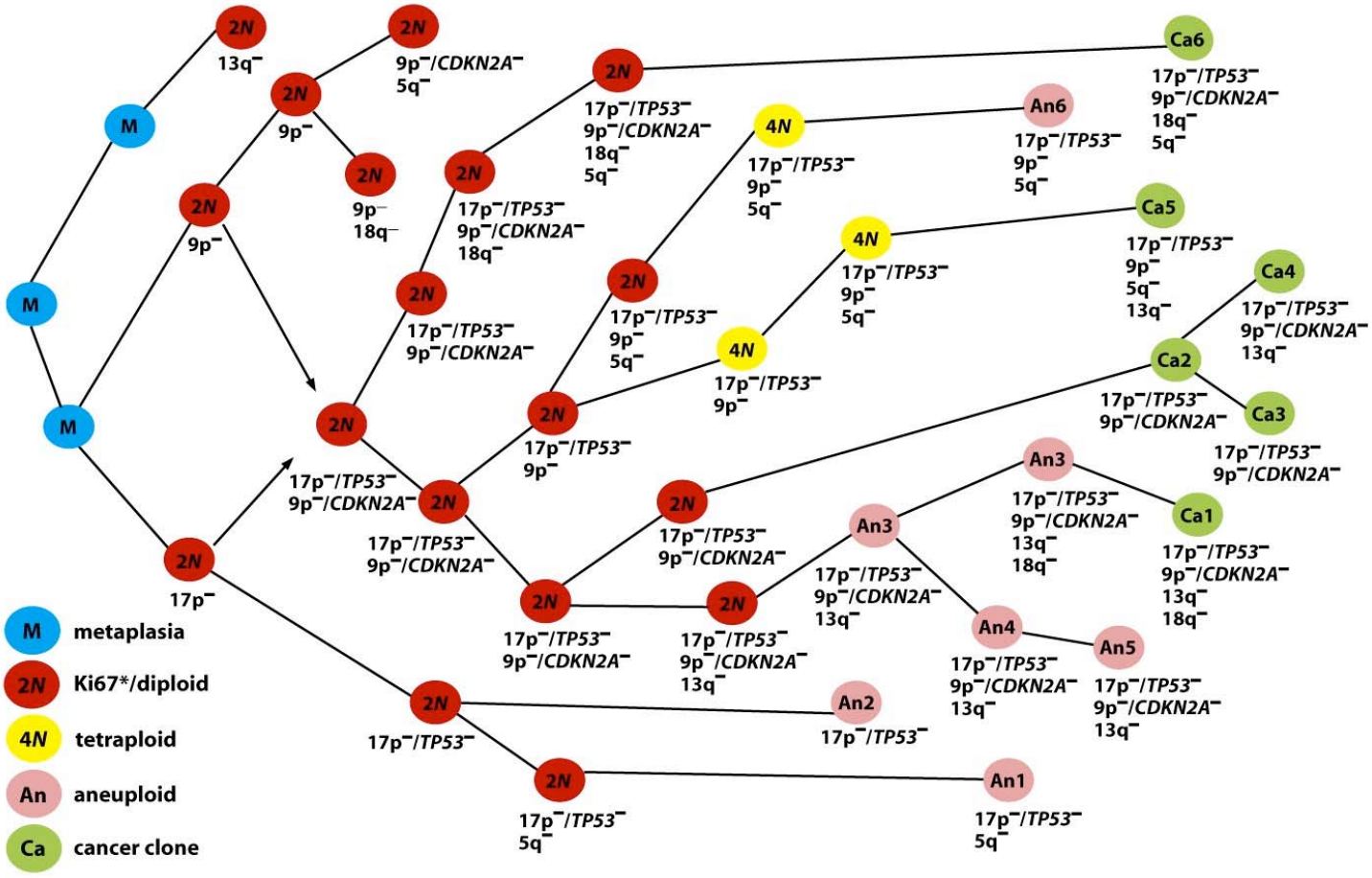
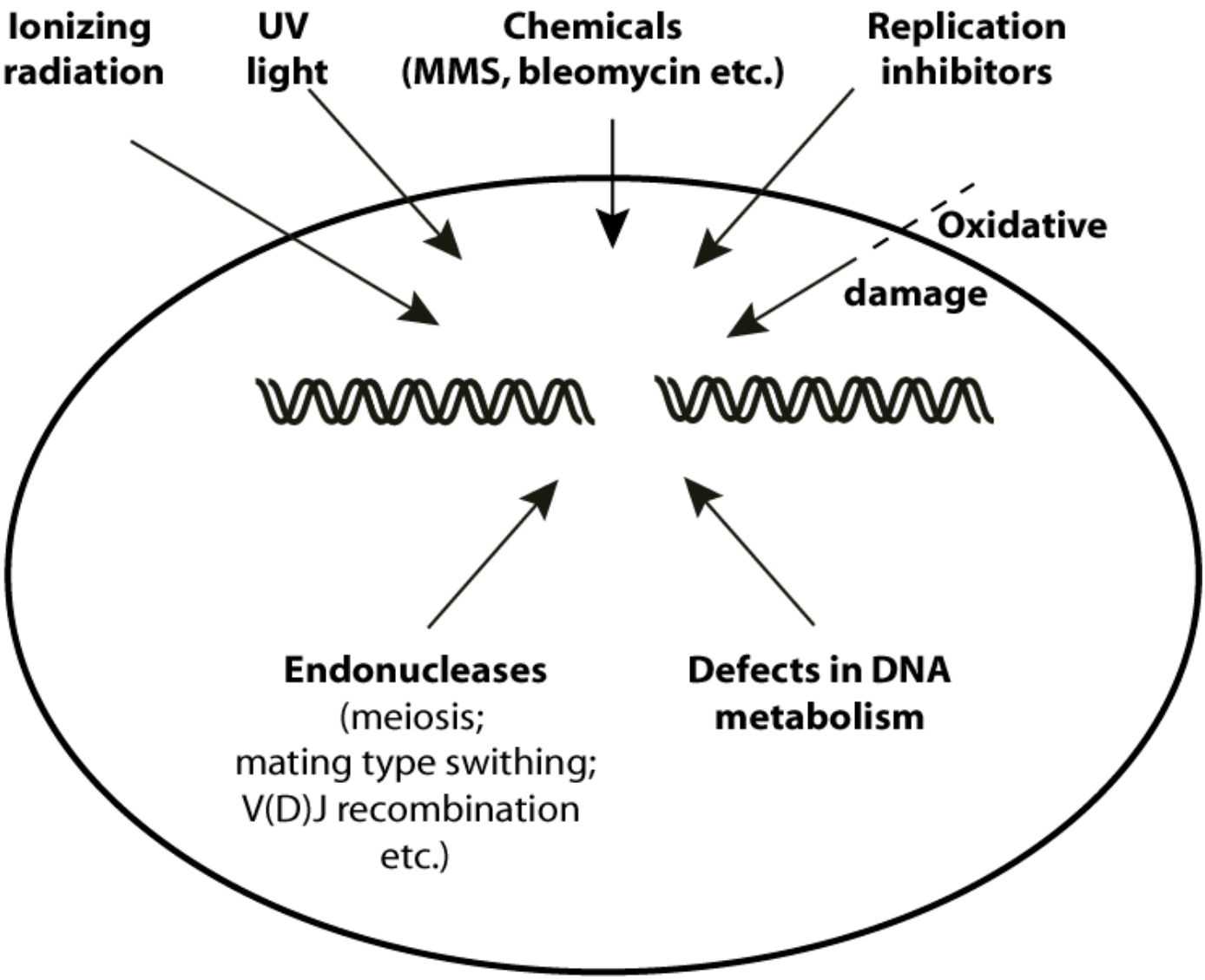
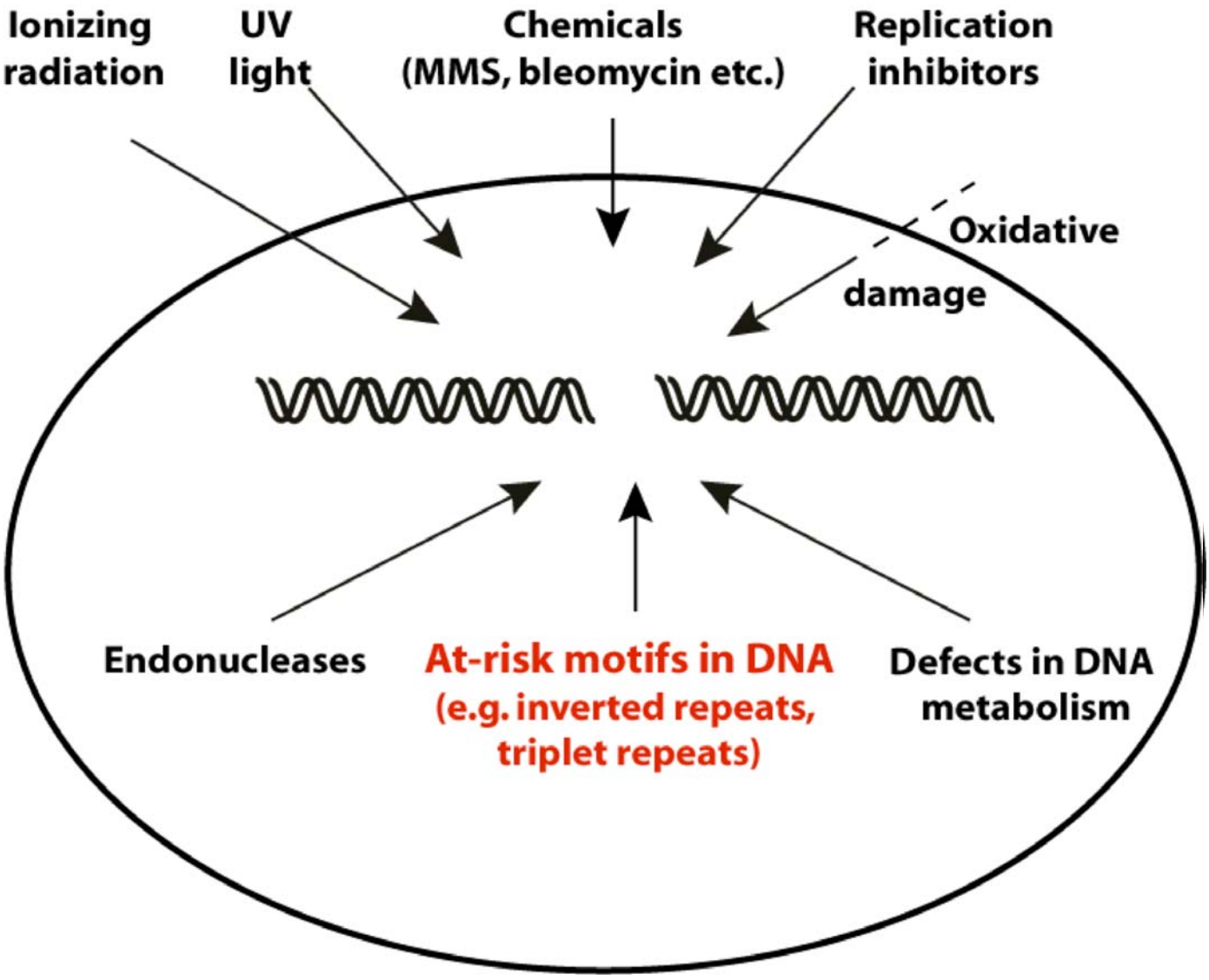


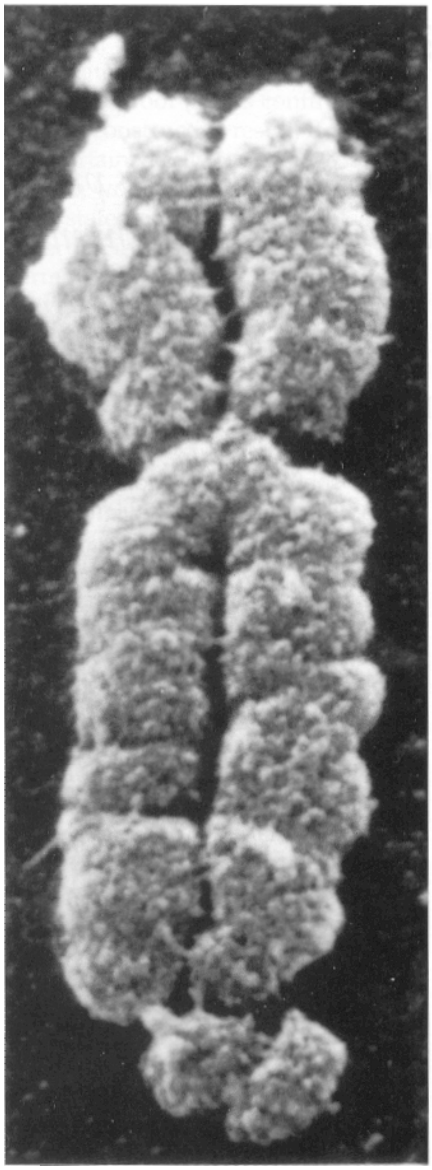
External and internal sources of chromosomal fragility

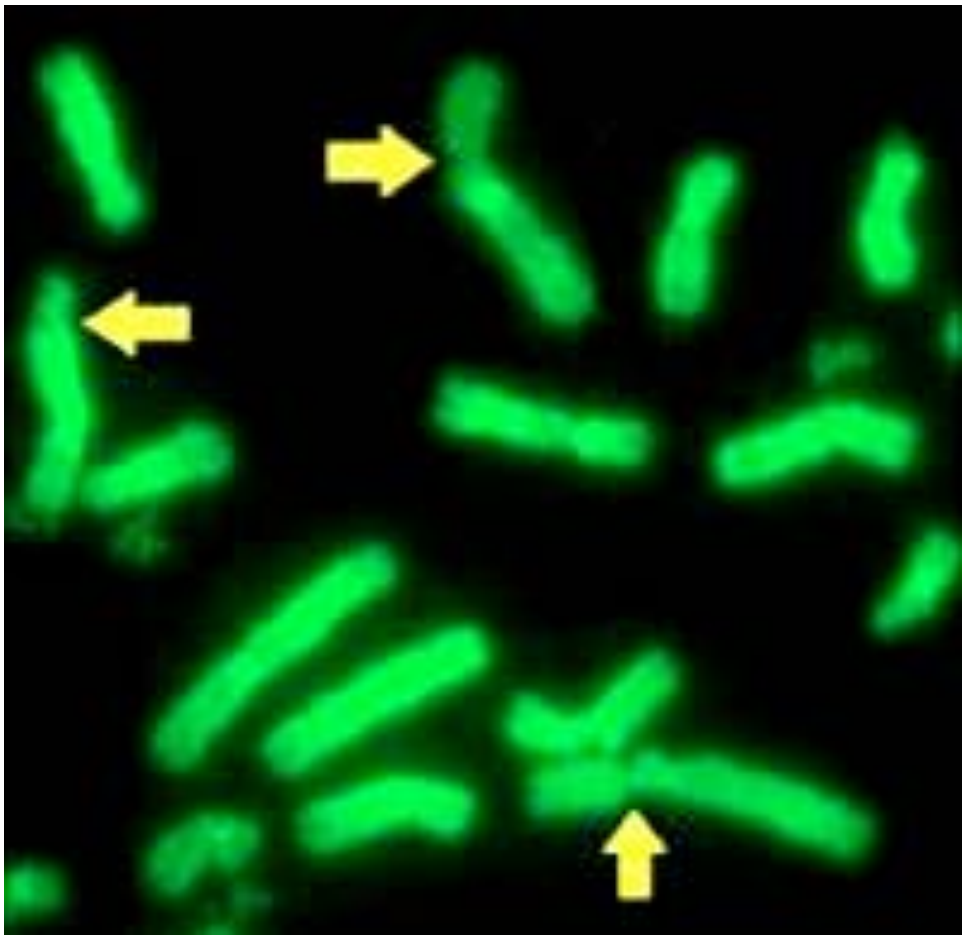


Repeats can be a source of fragility



Chromosomal fragility sites



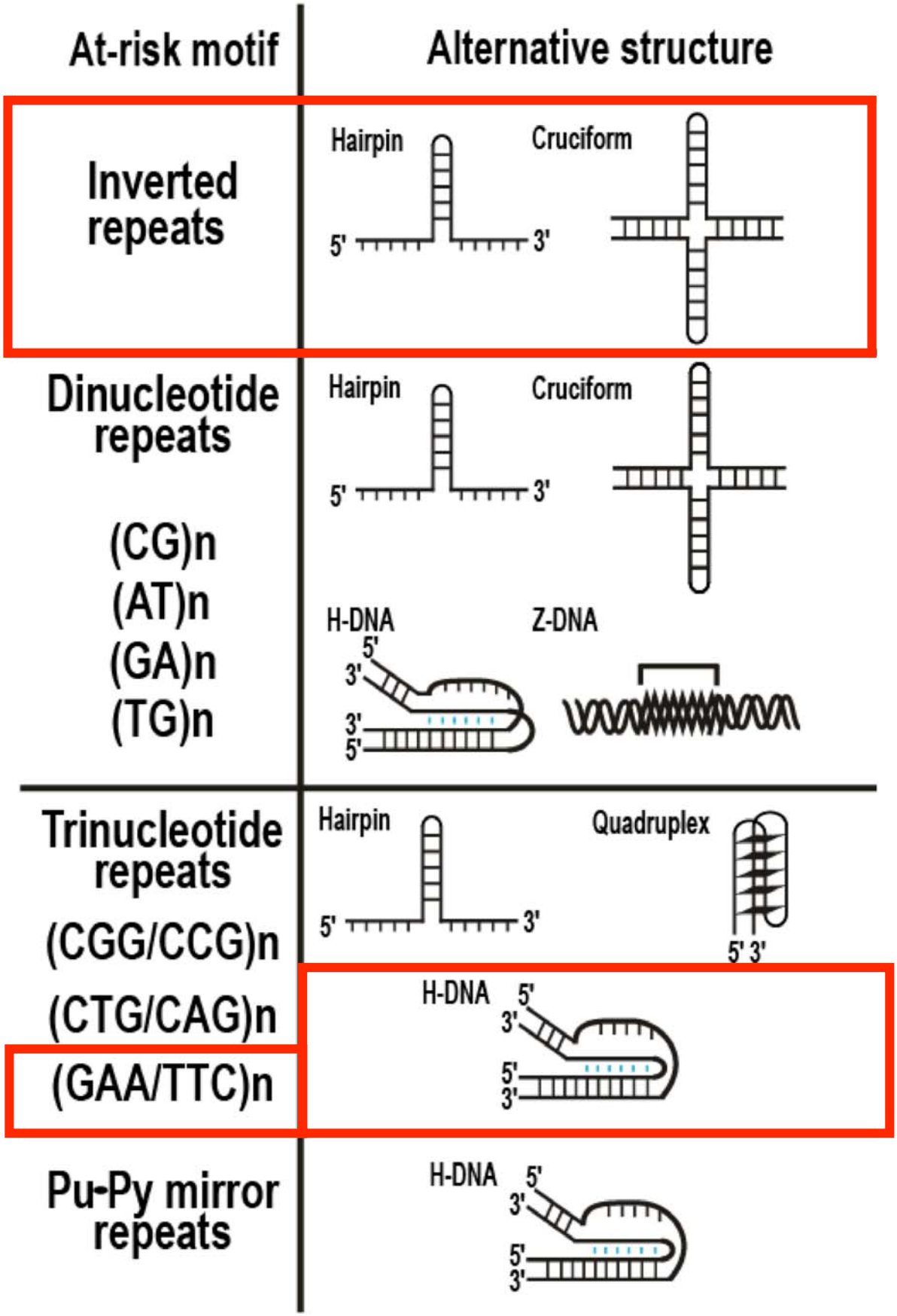


Fragile X

FRAXA, FRAXE, FRAXF, FRA11B, FRA16A, FRA10A are "rare" folate sensitive fragile sites occurring due to *CGG* expansions

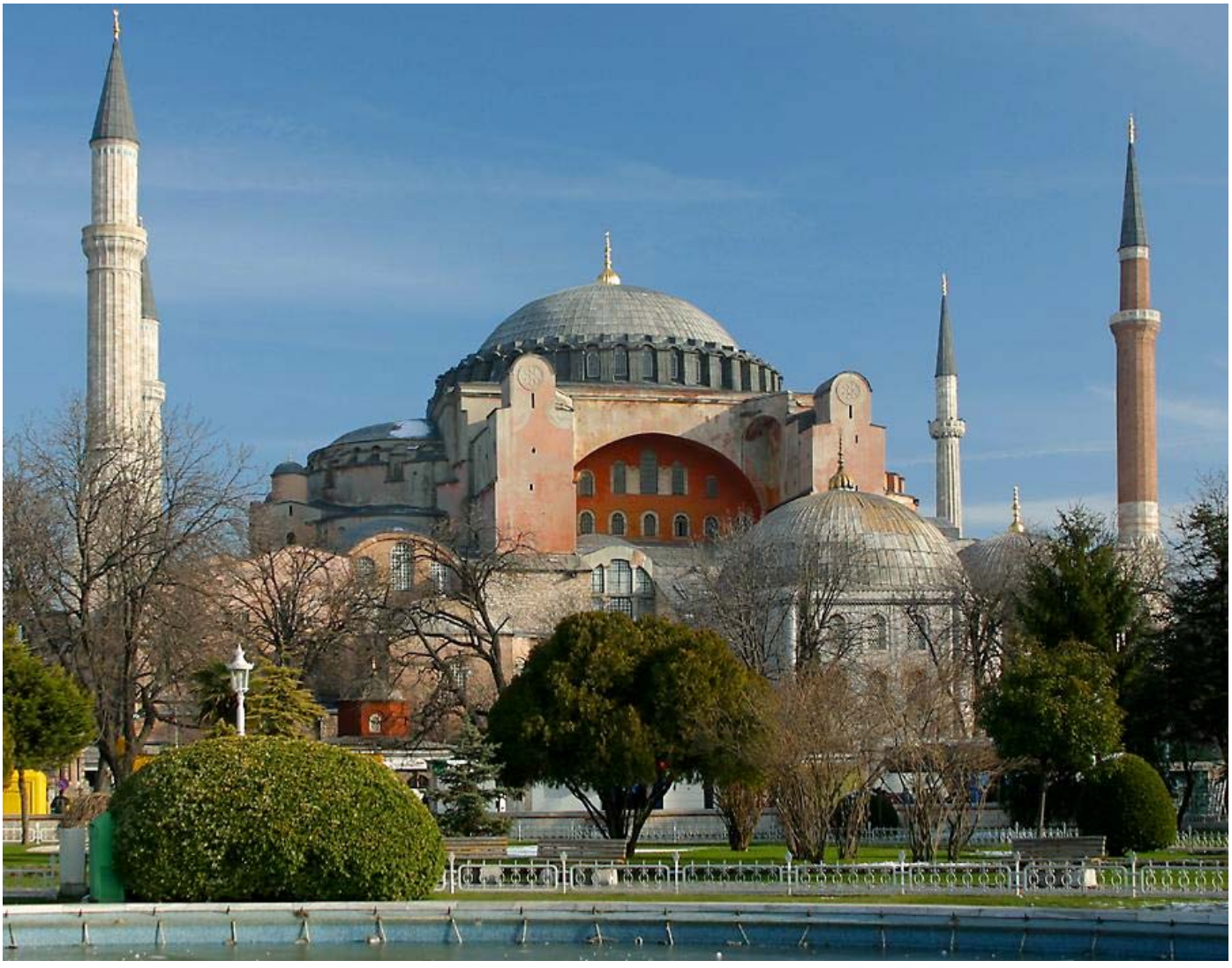
FRA10B and FRA16B are comprised of AT-rich minisatellites

At-risk motifs



Palindromes and genetic instability

NIYON ANOMHMATA MH MONAN OYIN

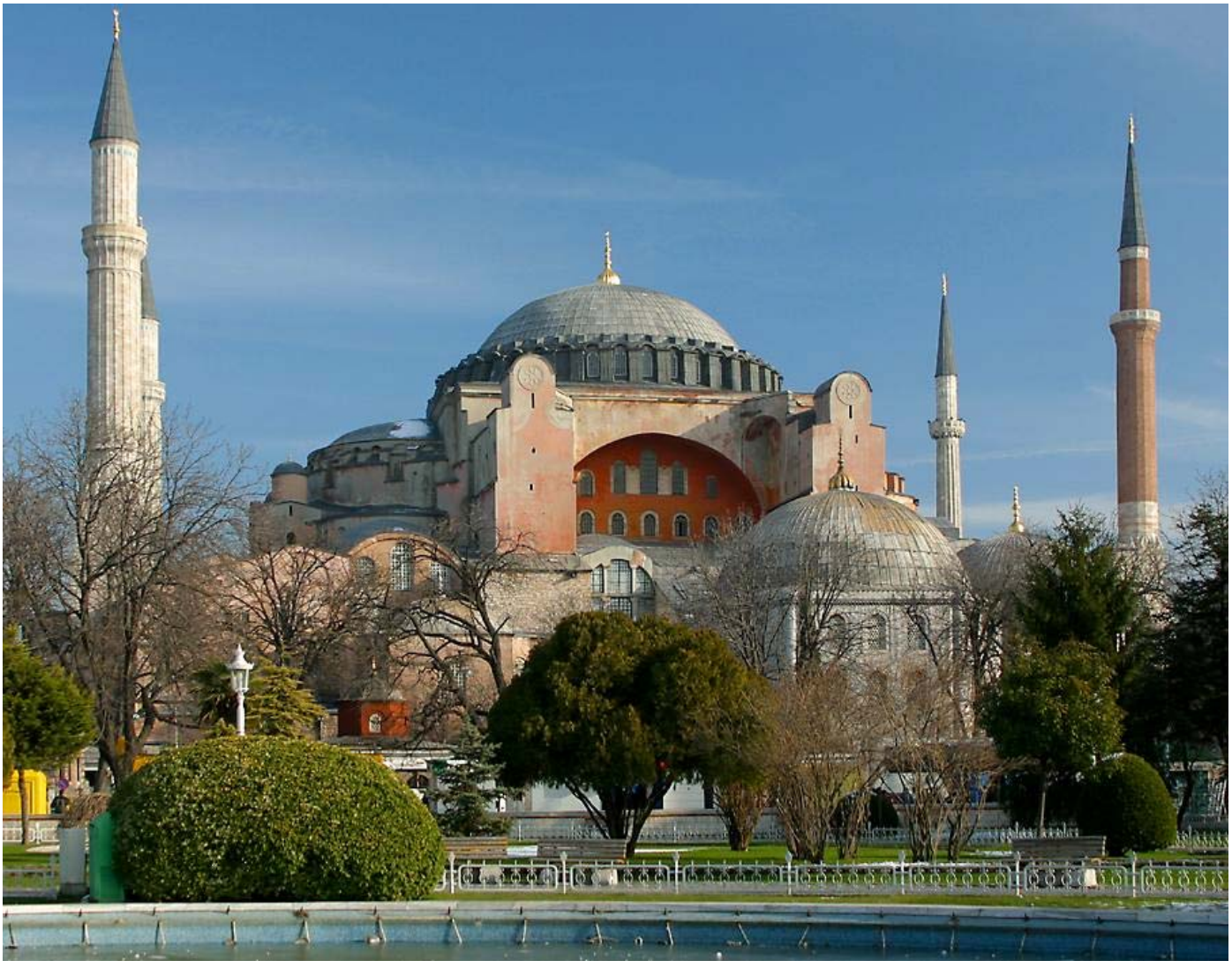


Hagia Sofia (537 AD)

Palindromes and genetic instability

NIYON ANOMHMATA MH MONAN OYIN

"Wash your sins, not just your face"



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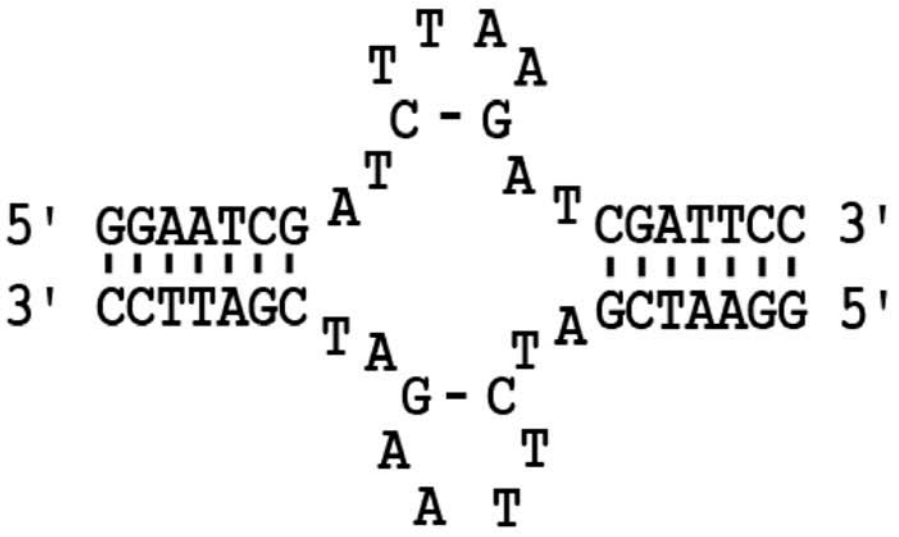

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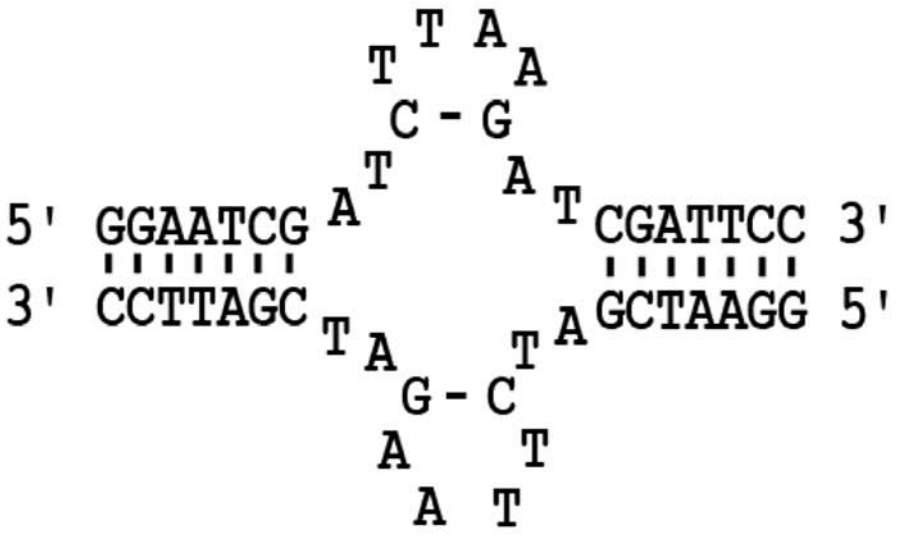
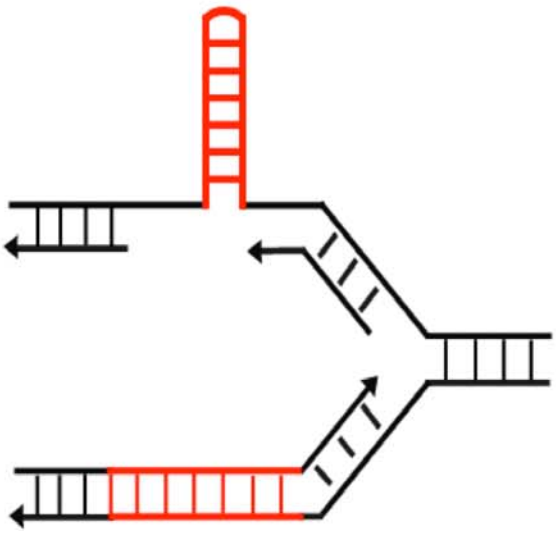
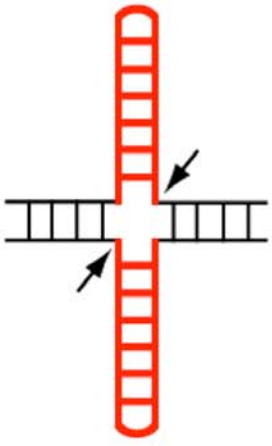


Replication fork collapse



Target for nucleases



Sources of repetitive sequences capable of adopting hairpin and cruciform structures

- **Inherently present in the human genome**

  (e.g. Y *chromosome, ribosomal cluster, t(11;22), NF1)*

  Human DNA Palindrome Database at http://vhp.ntu.edu.sg/hpaldb

  Lu et al., Funct. Integr. Genomics 2007

- **Human cancer cell lines *enriched with palindromes***

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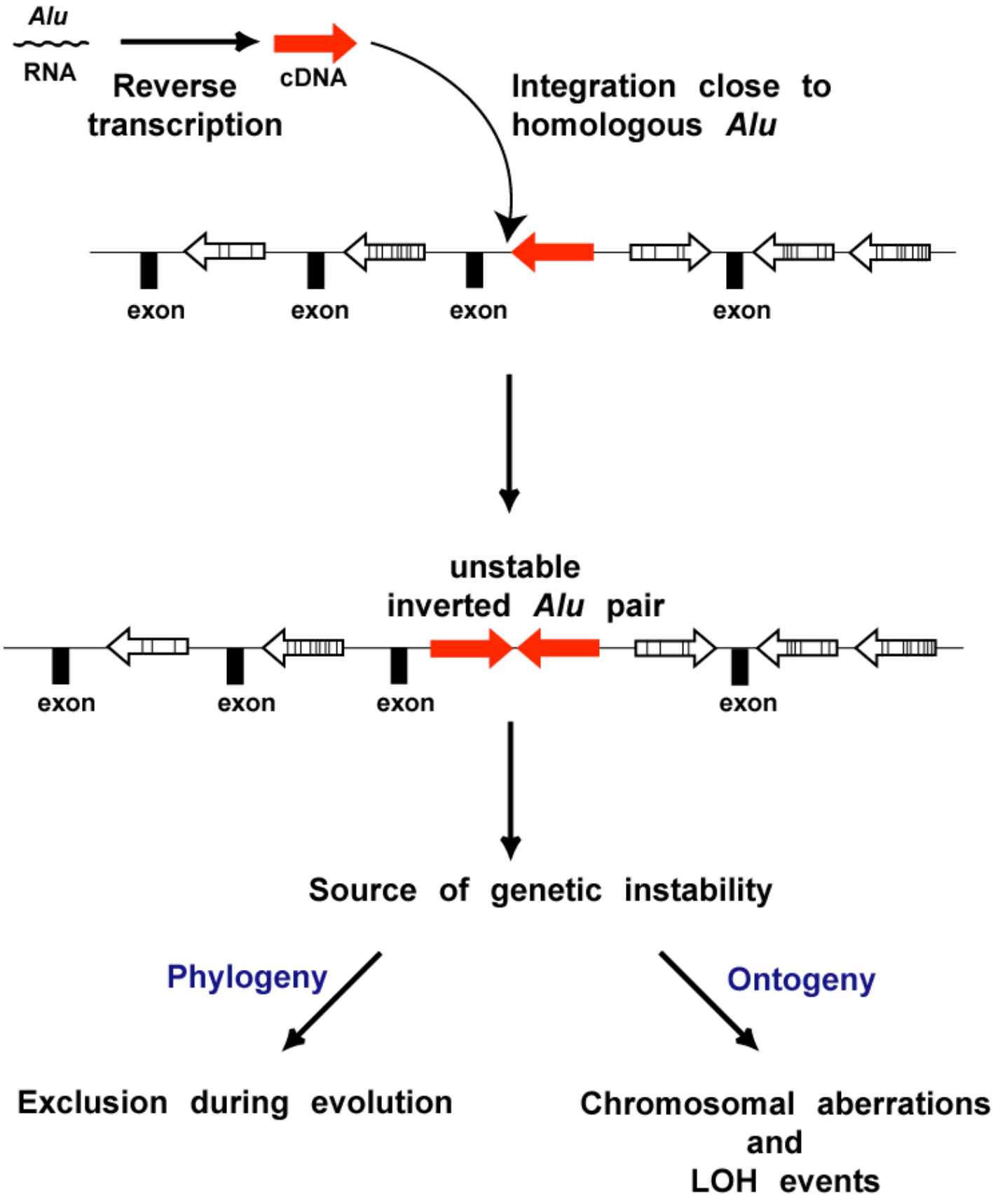

Lobachev et al EMBO 2000

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- **Transposition**

  (e.g. Alu elements)

- **Expansion of repeated sequences that have internal symmetry**

  (Tanaka et.al., Nature Genetics 2005)

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- **Transposition**

  (e.g. Alu elements)

- **Expansion of repeated sequences that have internal symmetry**

  Triplet repeats - CTG/CAG, CCG/CGG

  Di-nucleotide repeats AT or CG

- **Human cancer cell lines *enriched with palindromes***

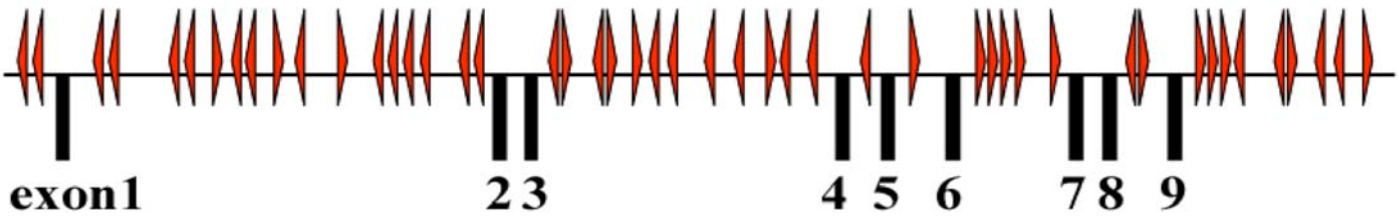
Alu repeats in the human genome

- ***Alu*** **average size: 300 bp;**
  Alu**s contain RNA polymerase III promoter element**

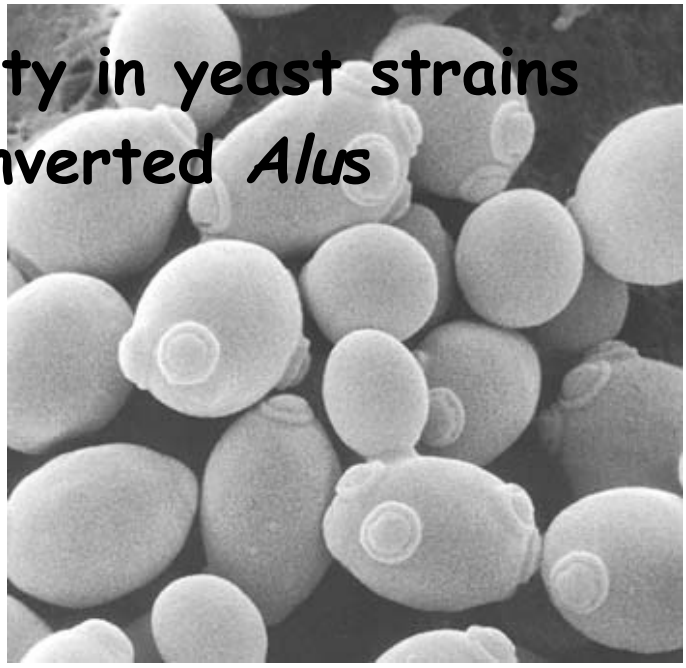
$$5' \text{ —— } (CpG)_n \text{ ——— } (A)_n \longrightarrow (A)_n\ 3'$$

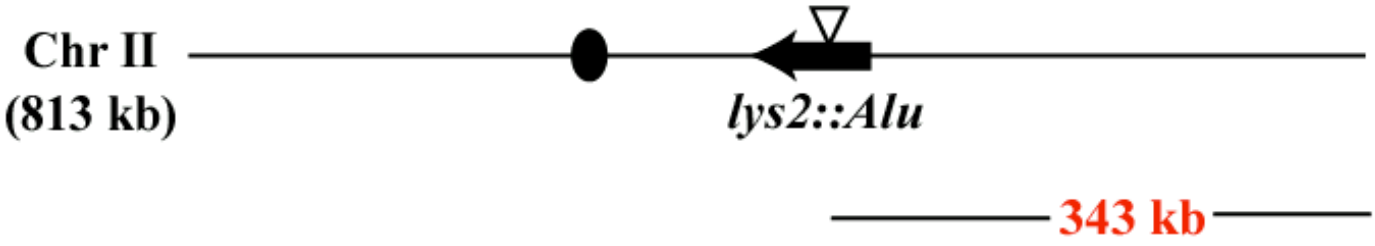
- **~ 1.1 x 10^6 copies of *Alu* repeats**
  (~10% of the human genome)
- ***Alu*** **repeats form clusters where they are closely-spaced**

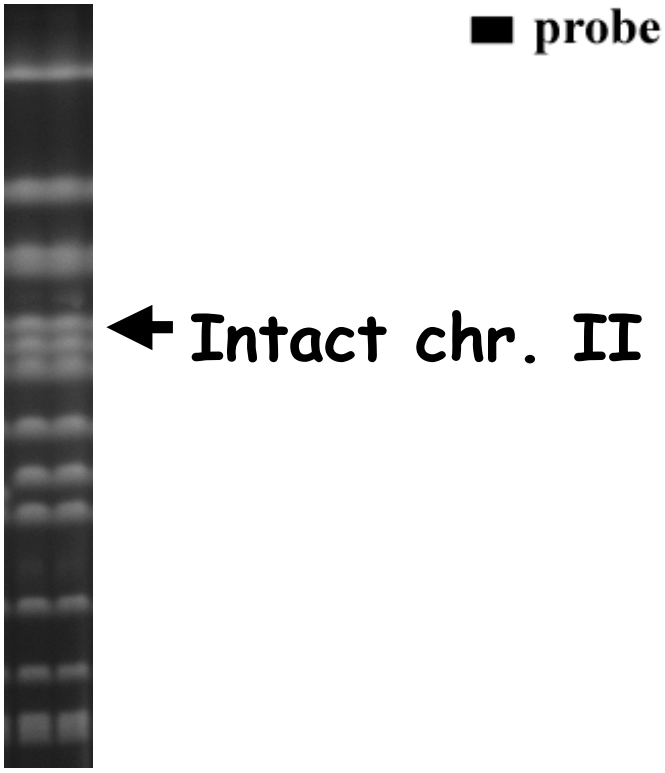
HPRT



Chromosomal fragility in yeast strains containing inverted *Alus*







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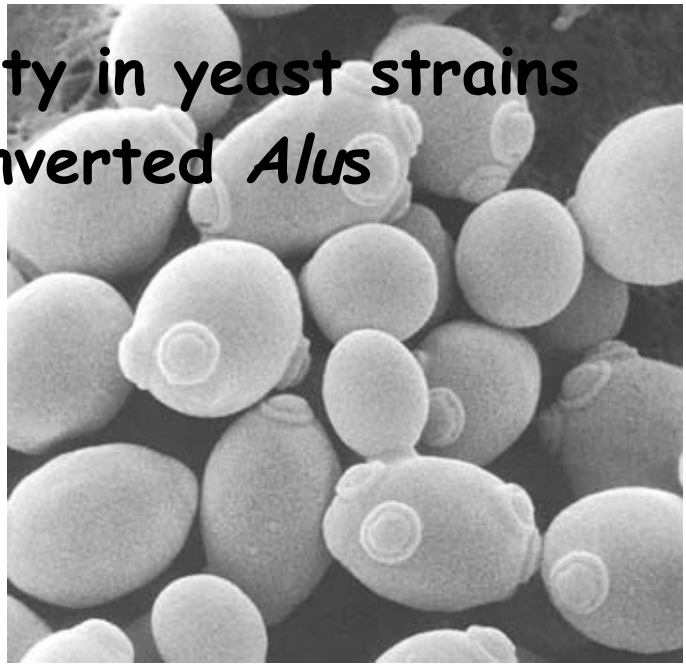
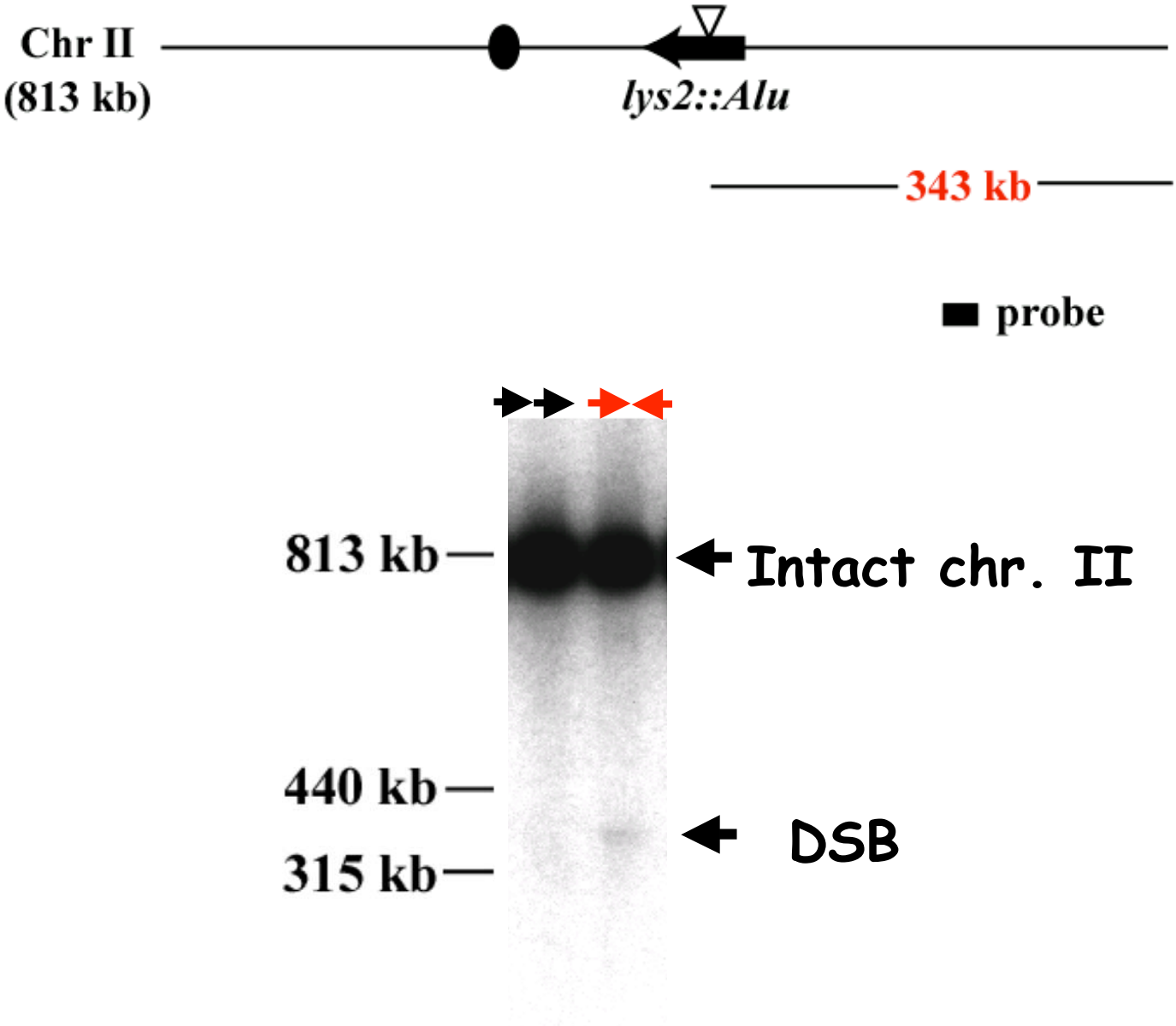
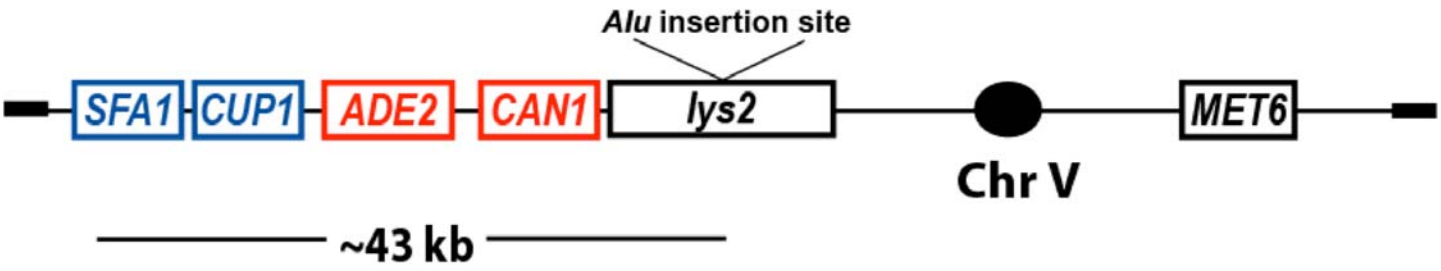




Experimental system to study chromosomal fragility induced by palindromic sequences



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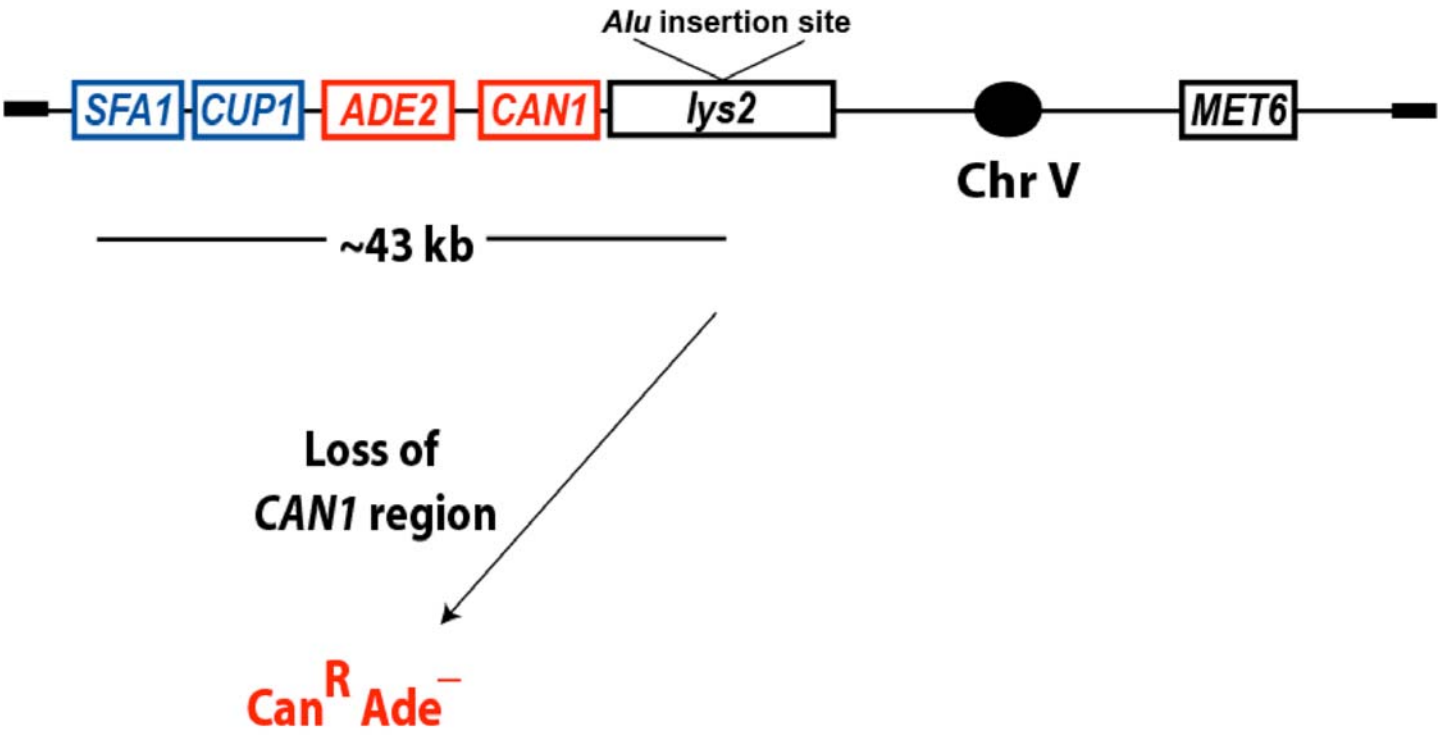

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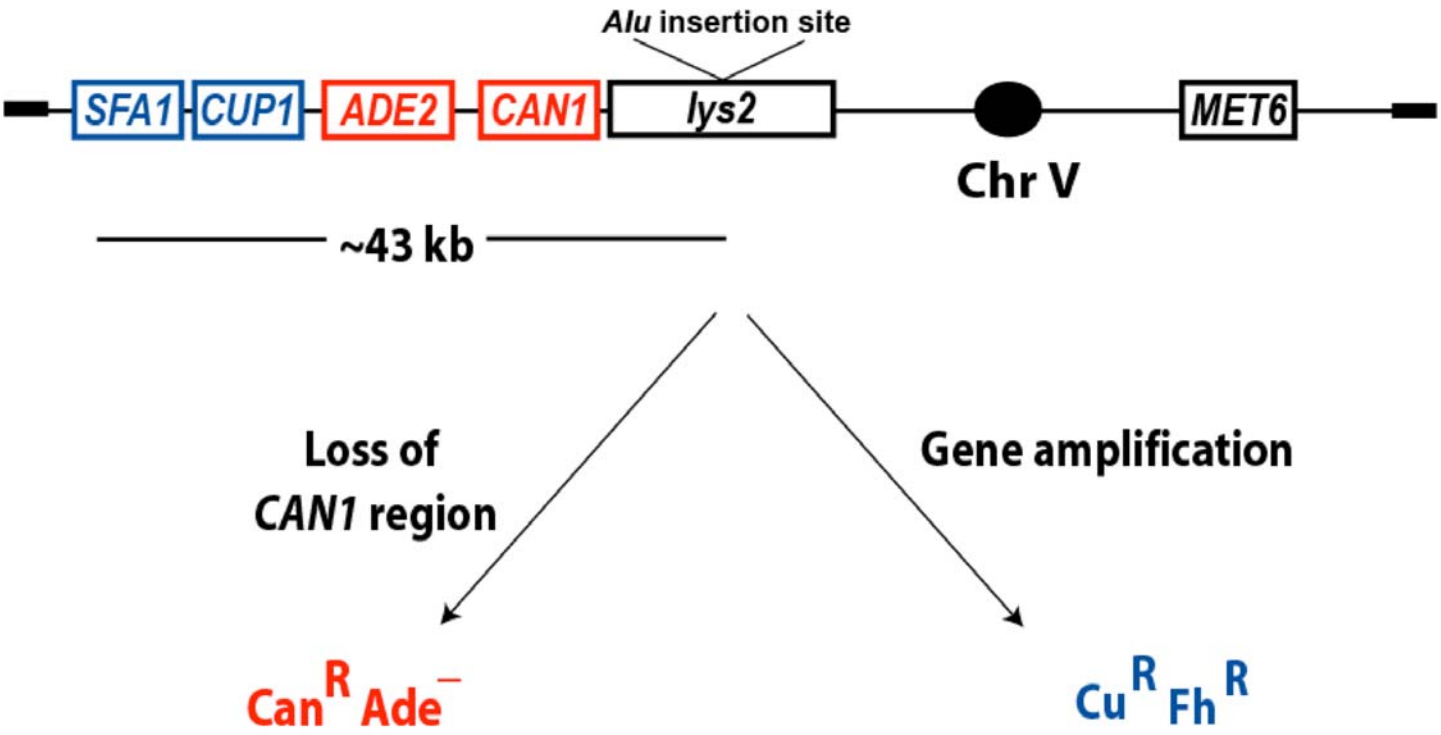

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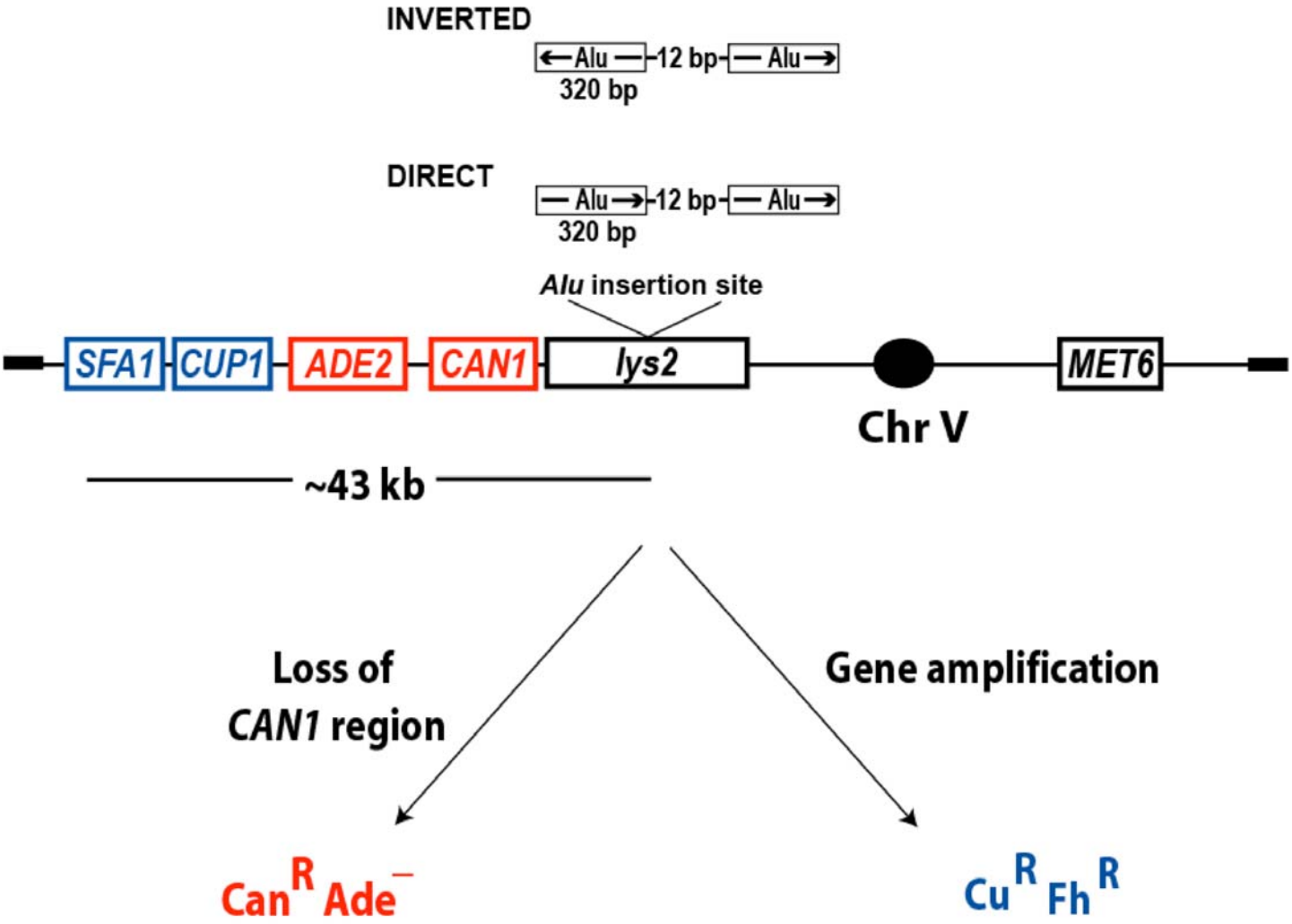

Inverted *Alu* repeats strongly induce chromosome V arm loss

		wt
Direct *Alus*		1x (1.5×10^{-9})
Inverted *Alus*	100%	25,000 x

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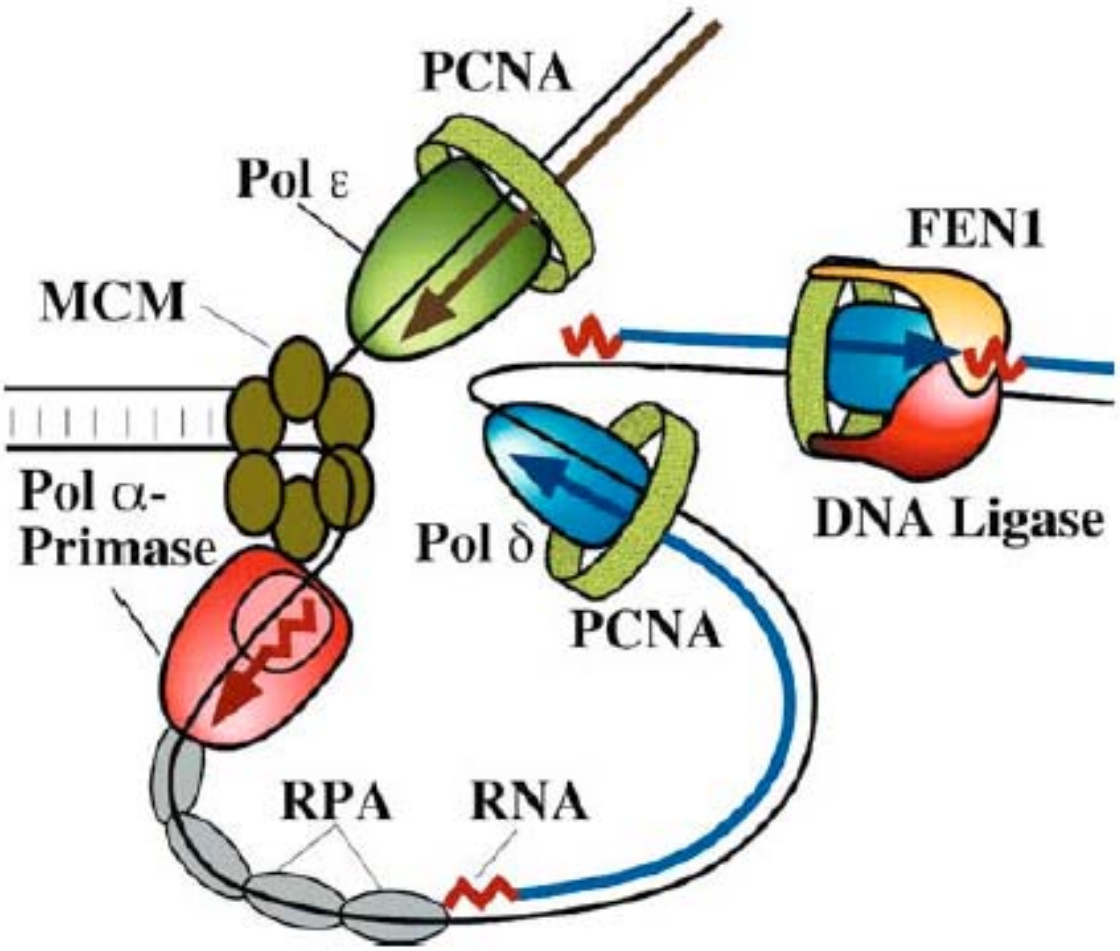
Inverted *Alus*	100%	25,000 x
	94%	1,800 x
	86%	470 x

Polymerase d mutants are prone for inverted repeat-mediated fragility

wt	1x
pol3-P664L	32x

Eukaryotic replication fork



Pursell et al., Science, 2007

Depletion of replication proteins increases inverted repeat–induced fragility

Lagging strand

wt	1x
pol3-P664L	32x
Tet-POL3 (d)	60x
Tet-POL1 (a)	77x
Tet-POL30 (PCNA)	65x

Depletion of replication proteins increases inverted repeat–induced fragility

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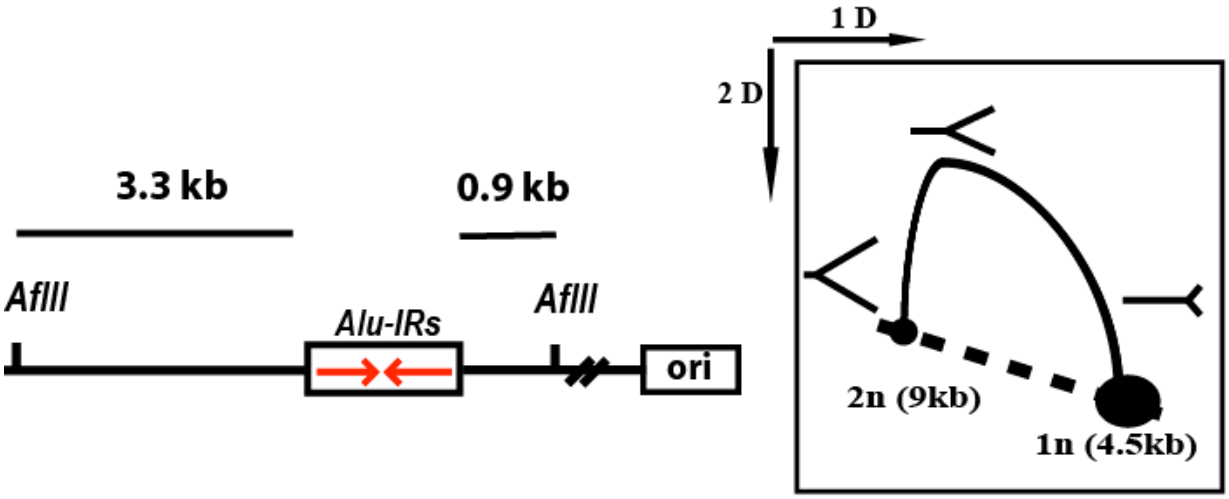
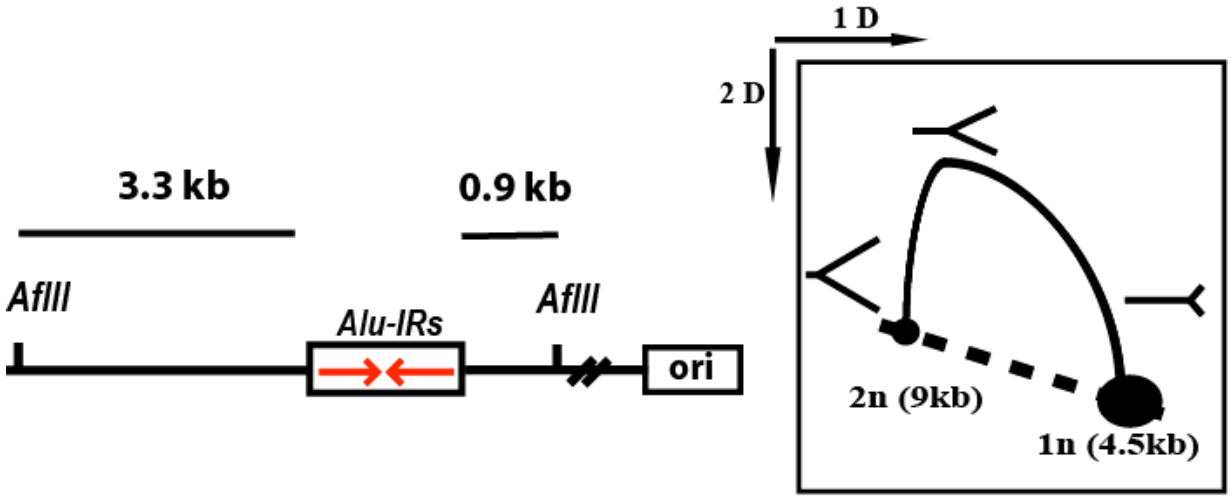
Lagging strand	*Tet-POL3 (d)*	60x
Lagging strand	*Tet-POL1 (a)*	77x
Lagging strand	*Tet-POL30 (PCNA)*	65x
Leading strand	*pol2-M644I*	26x
Leading strand	*Tet-POL2 (e)*	32x

2D analysis of the replication fork progression

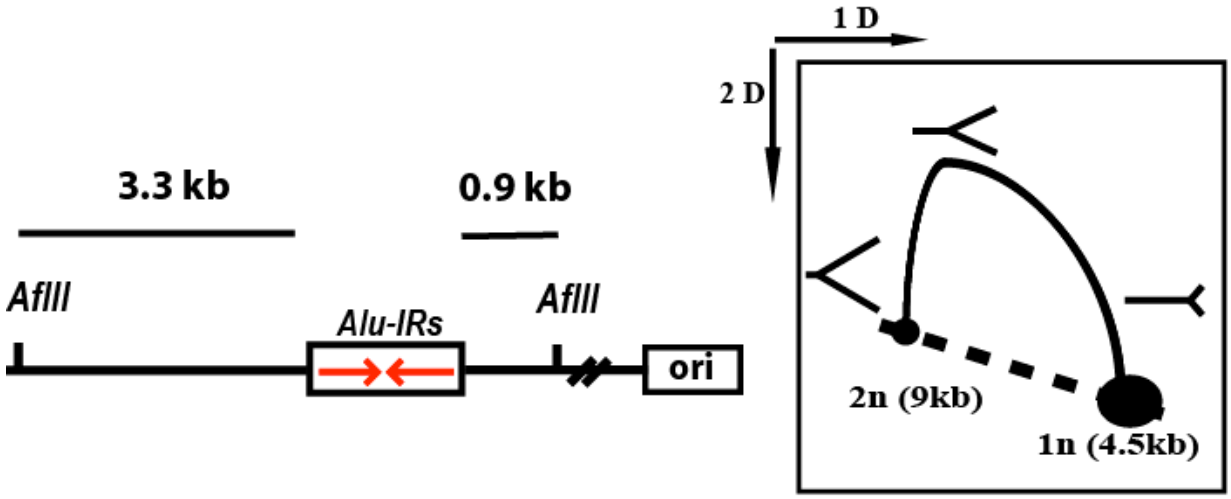


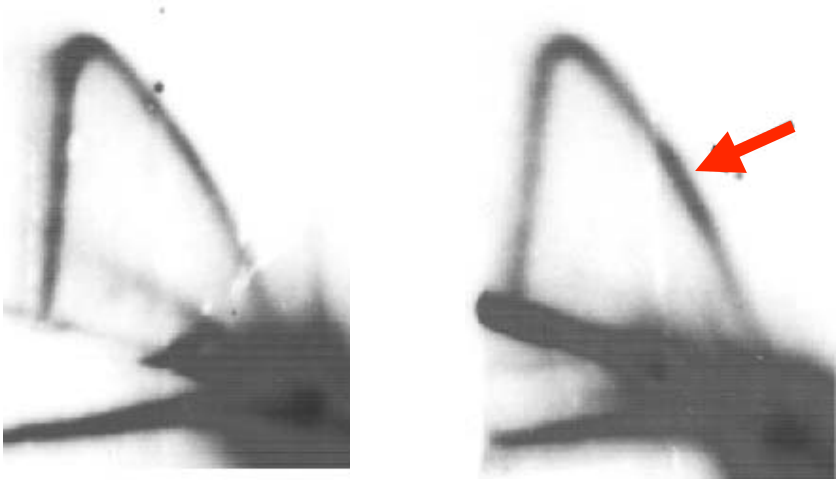
Inverted *Alu*s block replication progression



Wild type

Inverted *Alu*s block replication progression





дикий тип *pol3-P644L*

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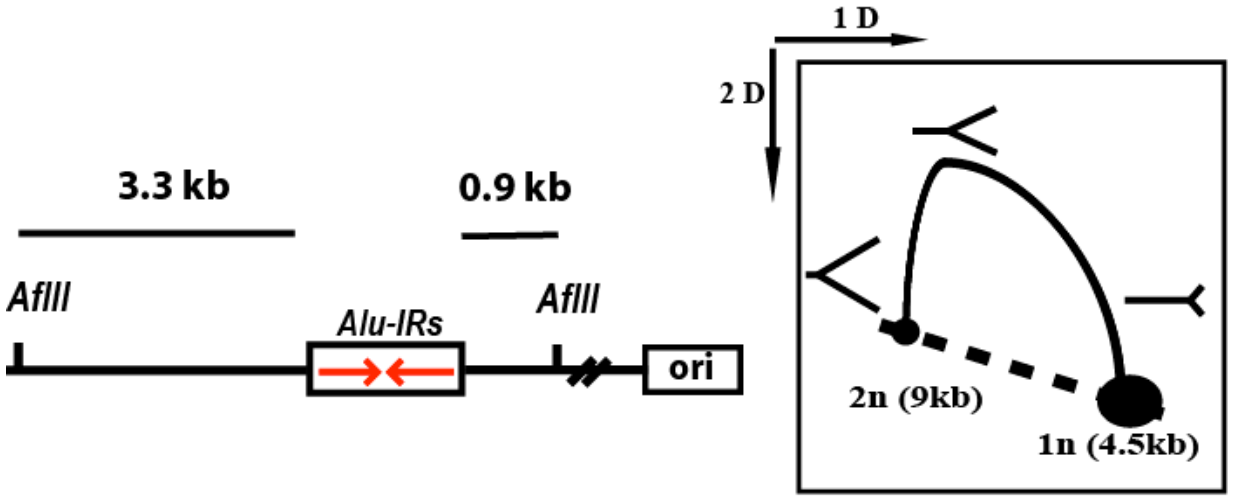
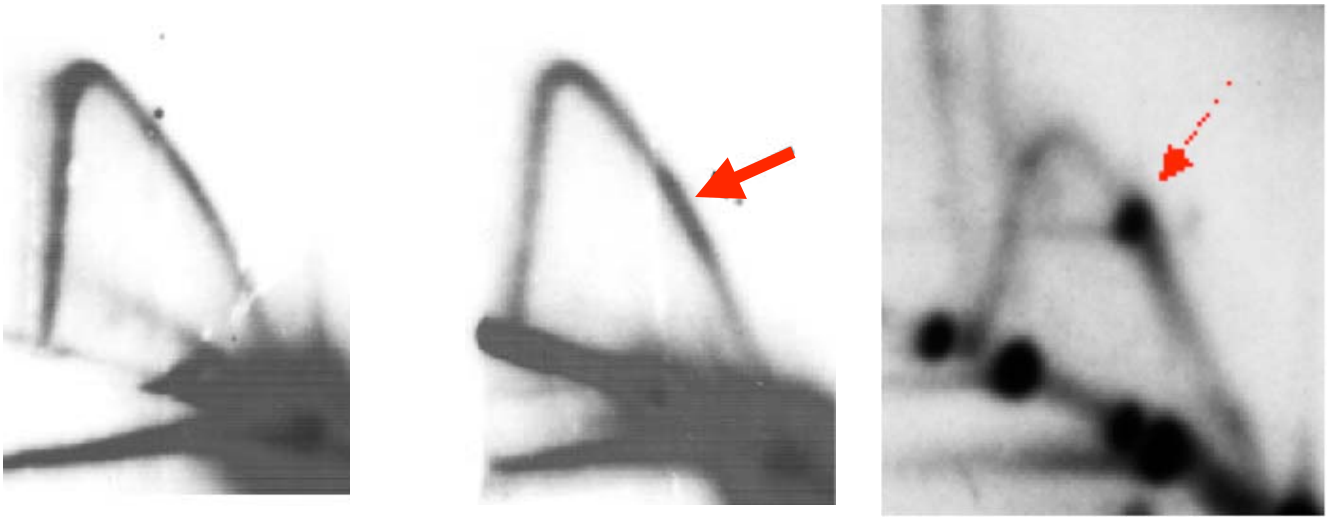
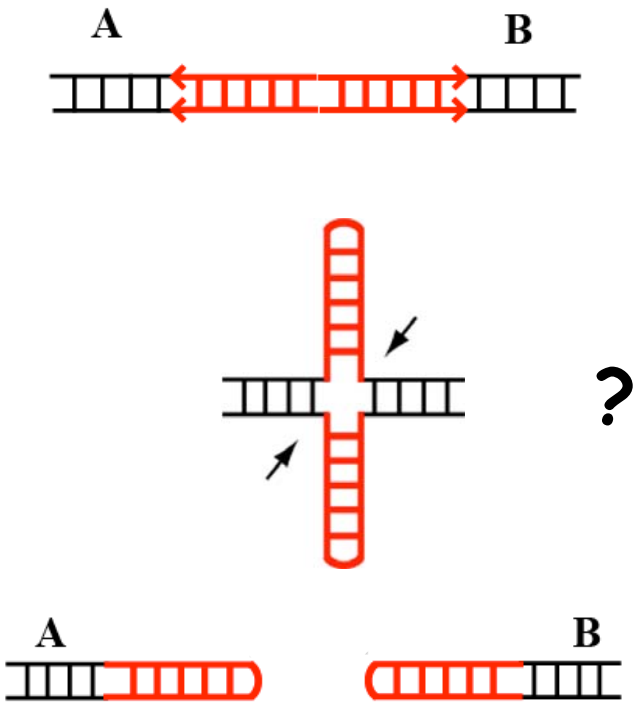




дикий тип *pol3-P644L* *E. coli*

Long quasi-palindromes induce hairpin-capped breaks



Lobachev et al, Cell 2002

Long quasi-palindromes induce hairpin-capped breaks

Does not depend on

Mus81/Mms4

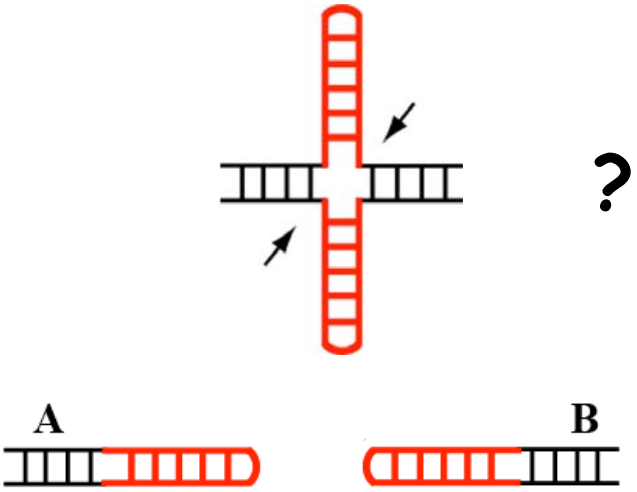
Slx1/Slx4

Rad1/Rad10

Top1

Top2

Top3



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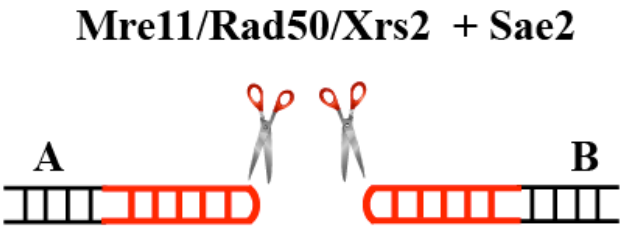

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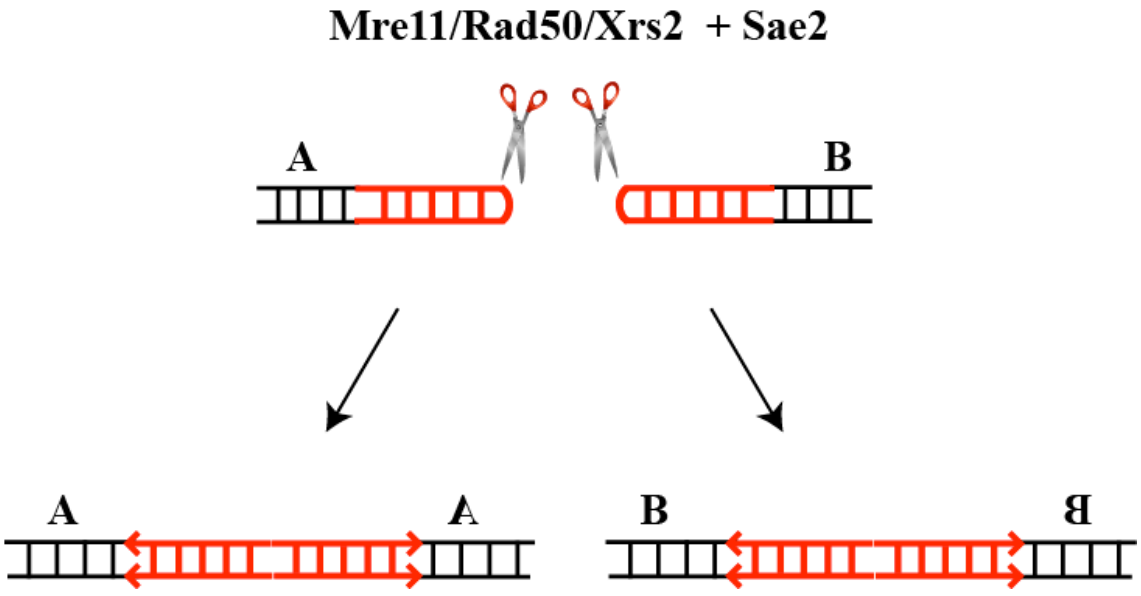
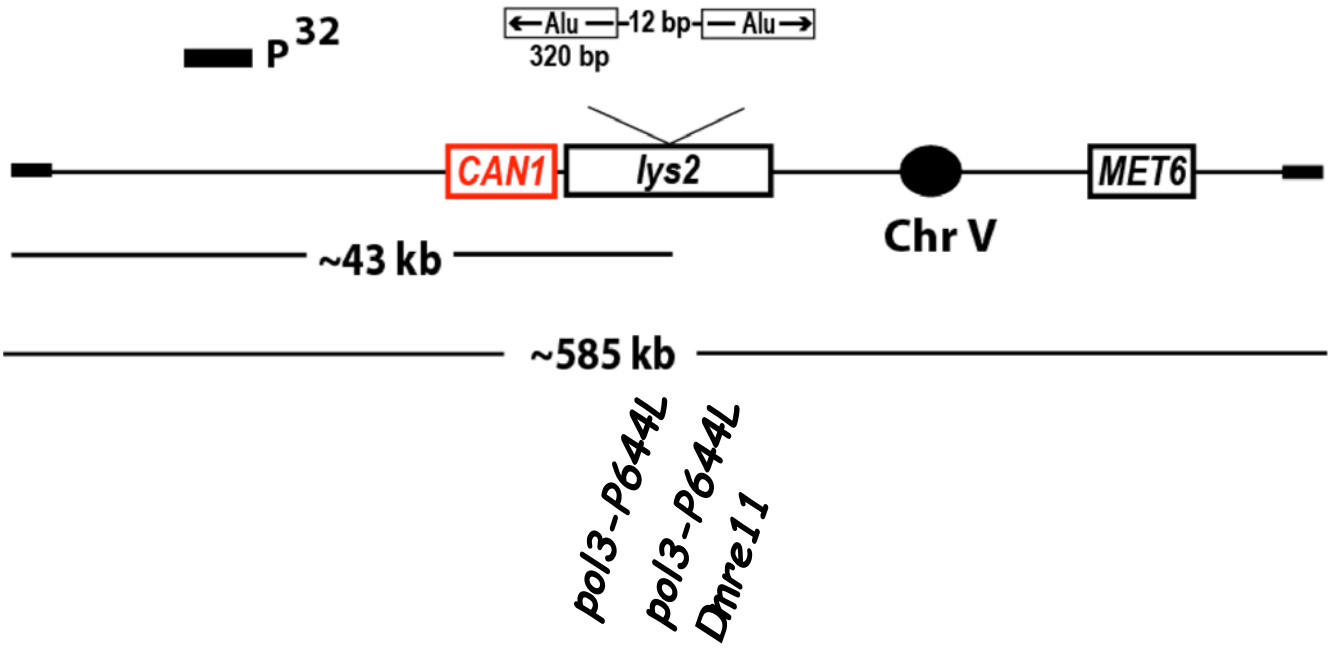
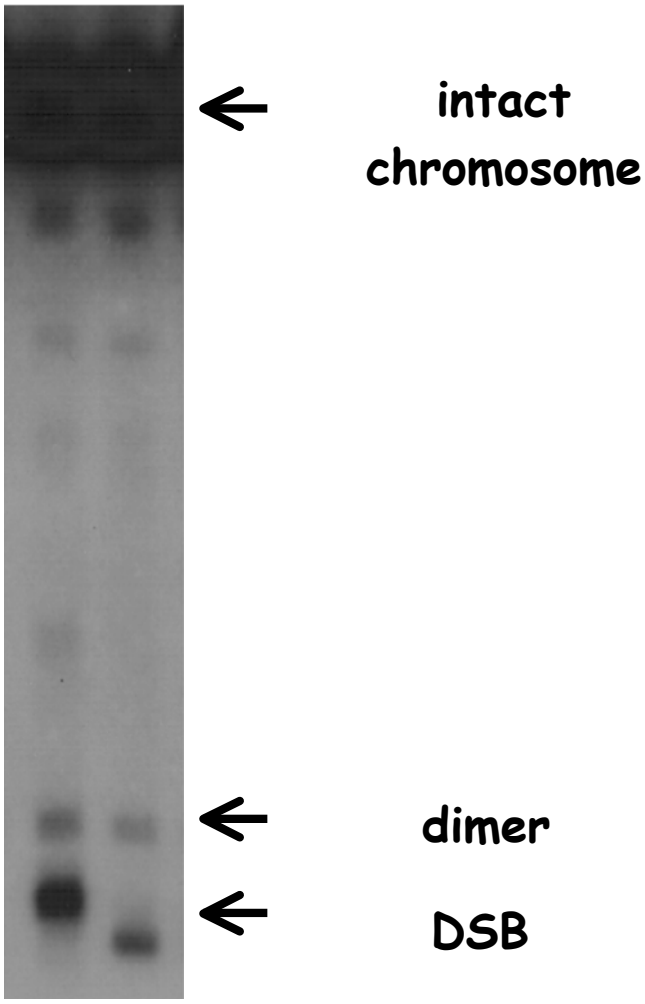


Lobachev et al, Cell 2002

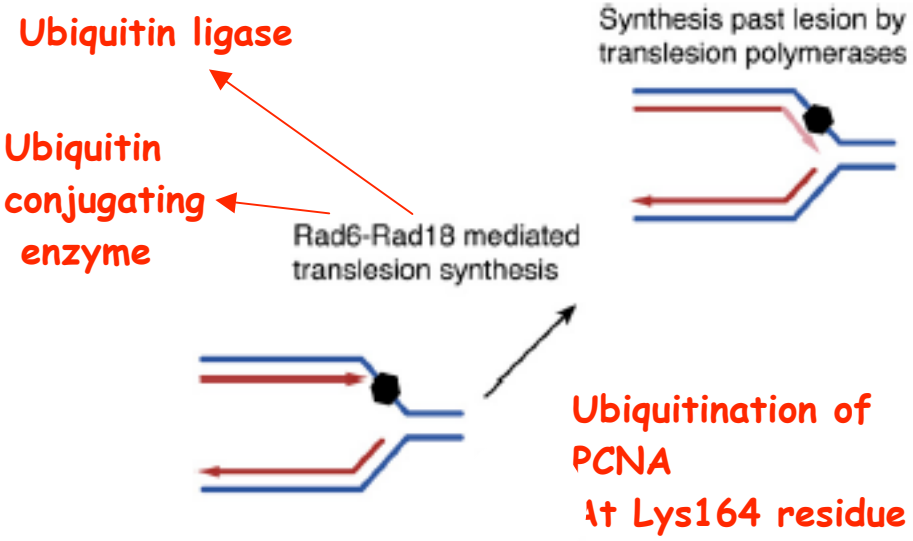
DSB formation in *pol3-P644L* strains with inverted *Alu*s





How and when the cruciform structure is formed?

DNA damage tolerance and template switching by post replicative repair (PRR)



Klein HL, Mol. Cell, 2007

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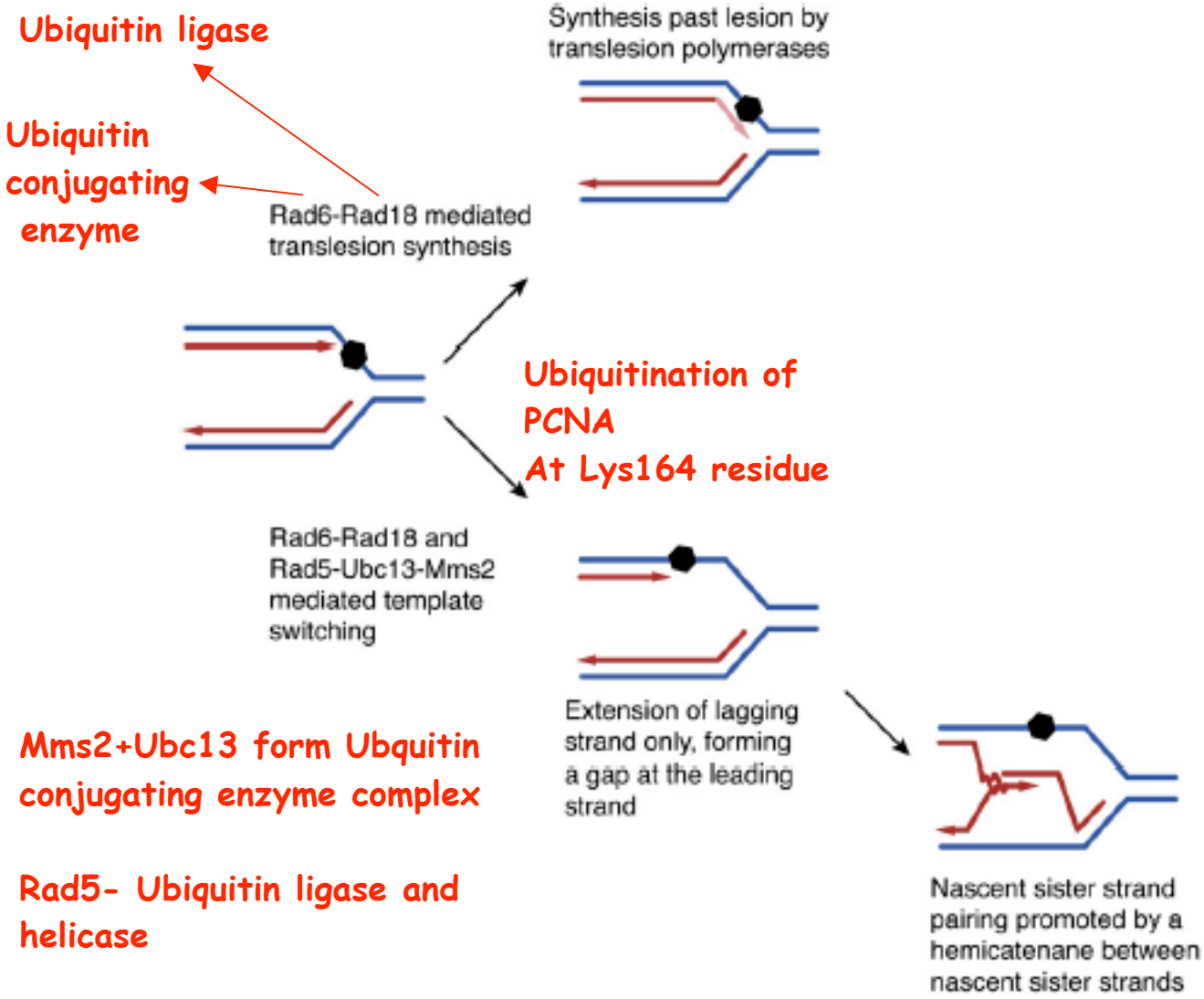

Mms2+Ubc13 form Ubquitin conjugating enzyme complex

Rad5- Ubiquitin ligase and helicase

Klein HL, Mol. Cell, 2007

Chromosome fragility due to compromised replication requires some components of post replicative repair (PRR)

wt	1x
pol3-P664L	32x
pol3-P664L rad5D	1x
pol3-P664L rad6D (rad18D)	1x
pol3-P664L pol30-K164R	30x
pol3-P664L ubc13D(mms2D)	35x

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pol3-P664L rad5D	1x
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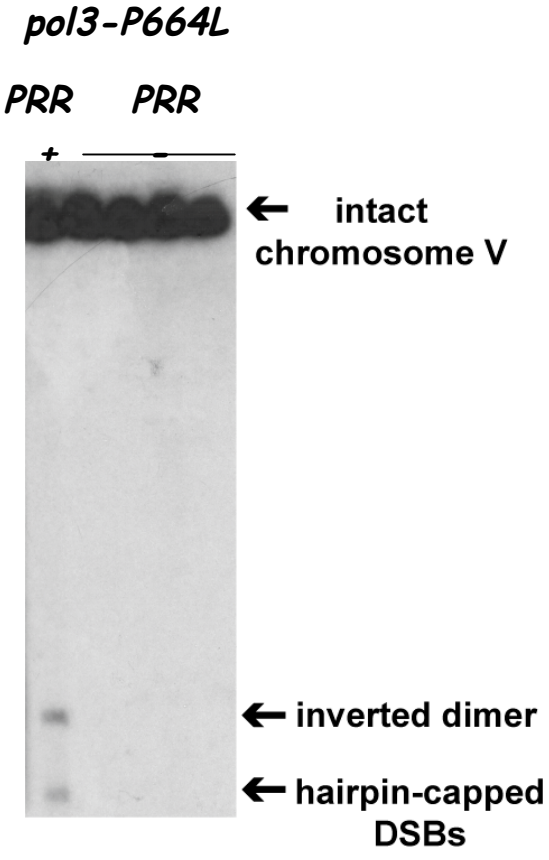
pol3-P664L pol30-K164R	30x
pol3-P664L ubc13D(mms2D)	35x



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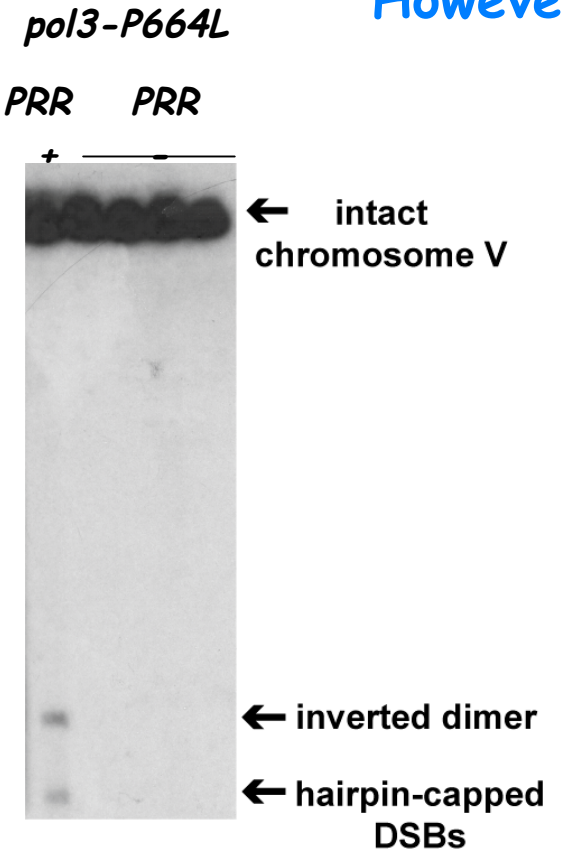
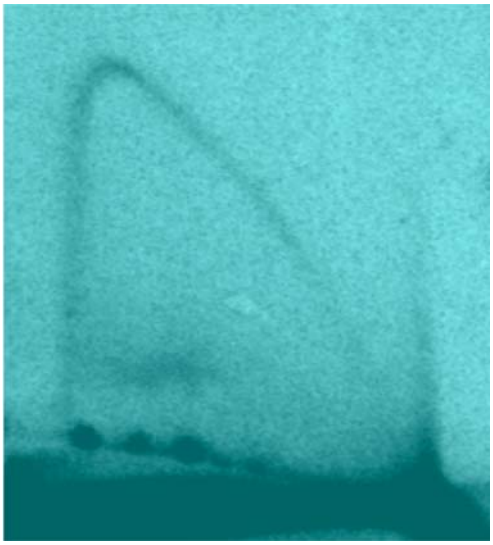
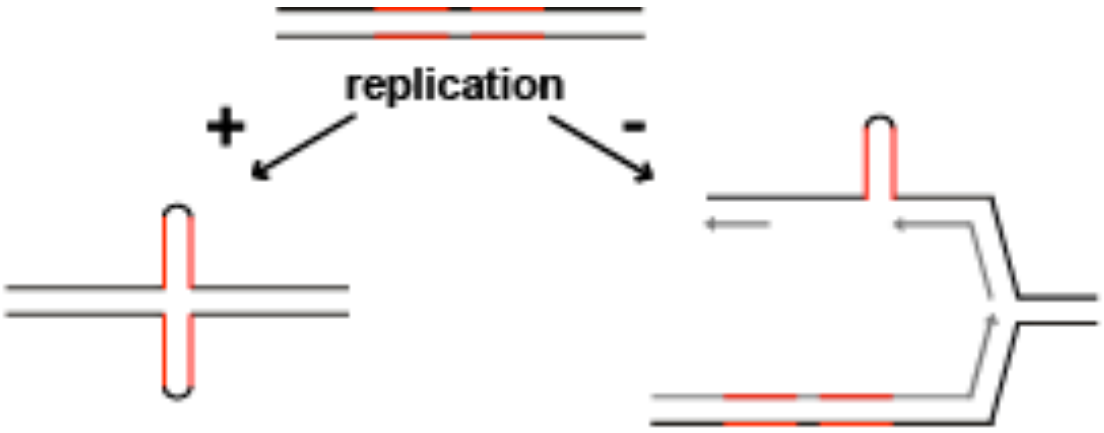
pol3-P664L	32x
pol3-P664L rad5D	1x
pol3-P664L rad6D (rad18D)	1x
pol3-P664L pol30-K164R	30x
pol3-P664L ubc13D(mms2D)	35x
However, *rad5D*	1x



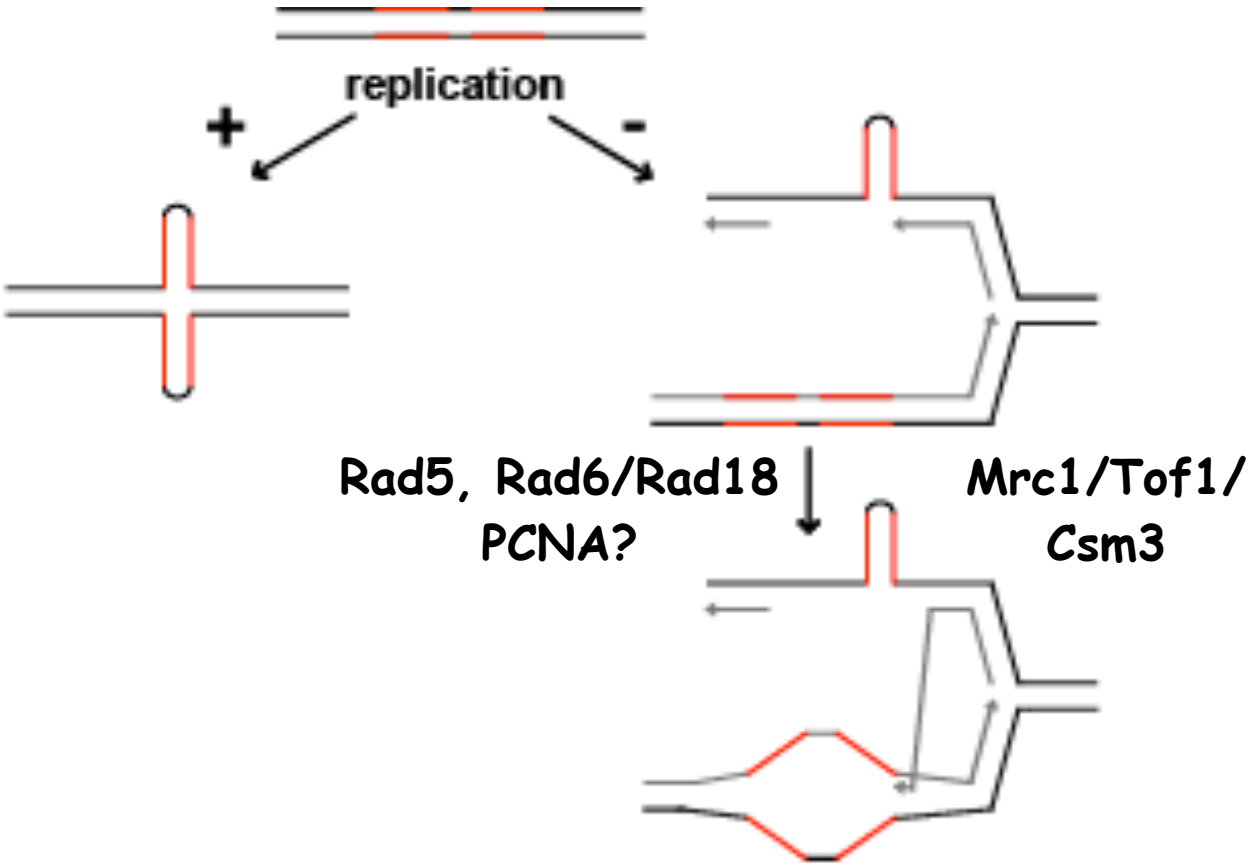


pol3-P664L rad5D

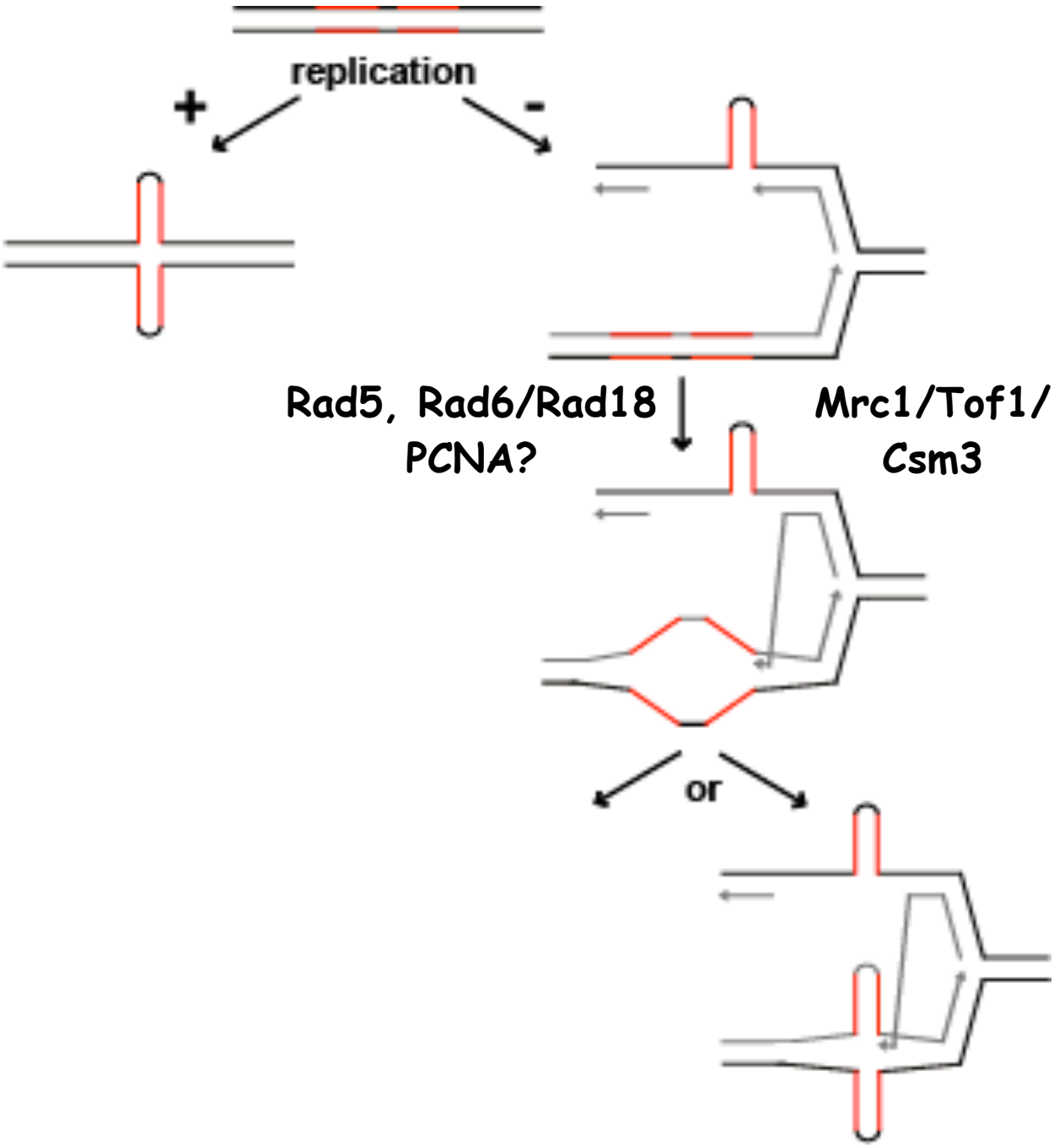
Working model for cruciform-mediated fragility



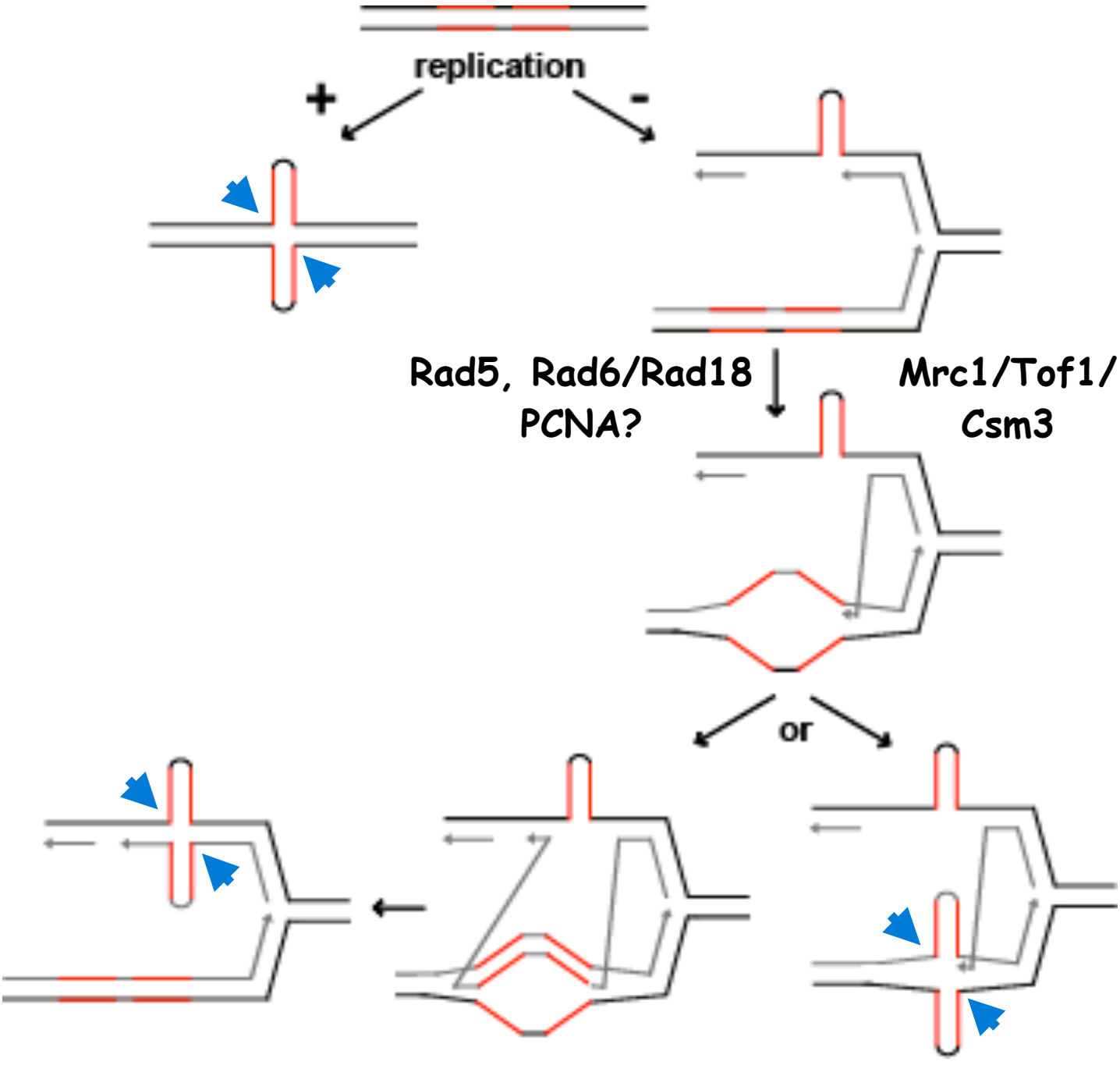
Working model for cruciform-mediated fragility



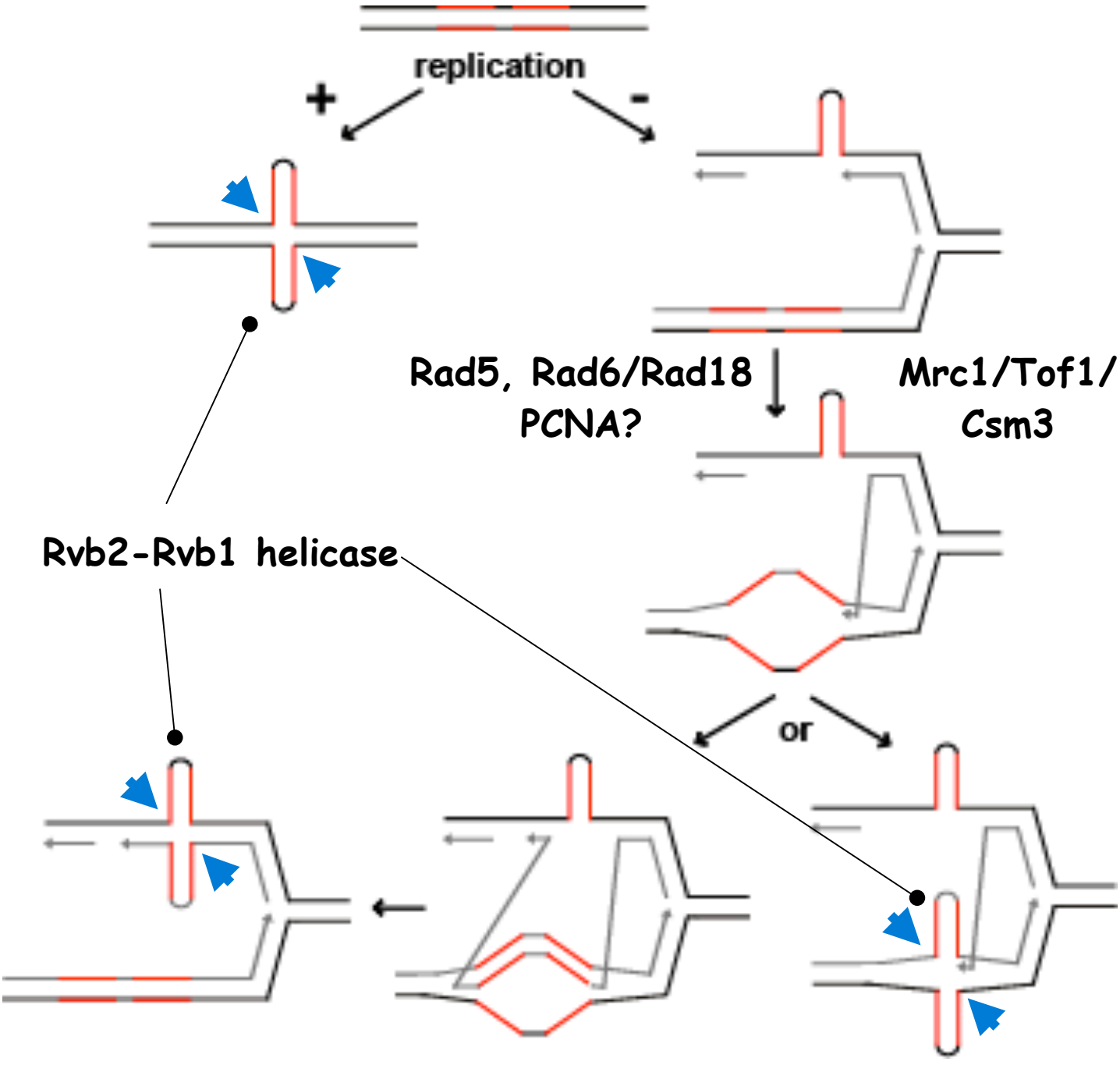
Working model for cruciform-mediated fragility



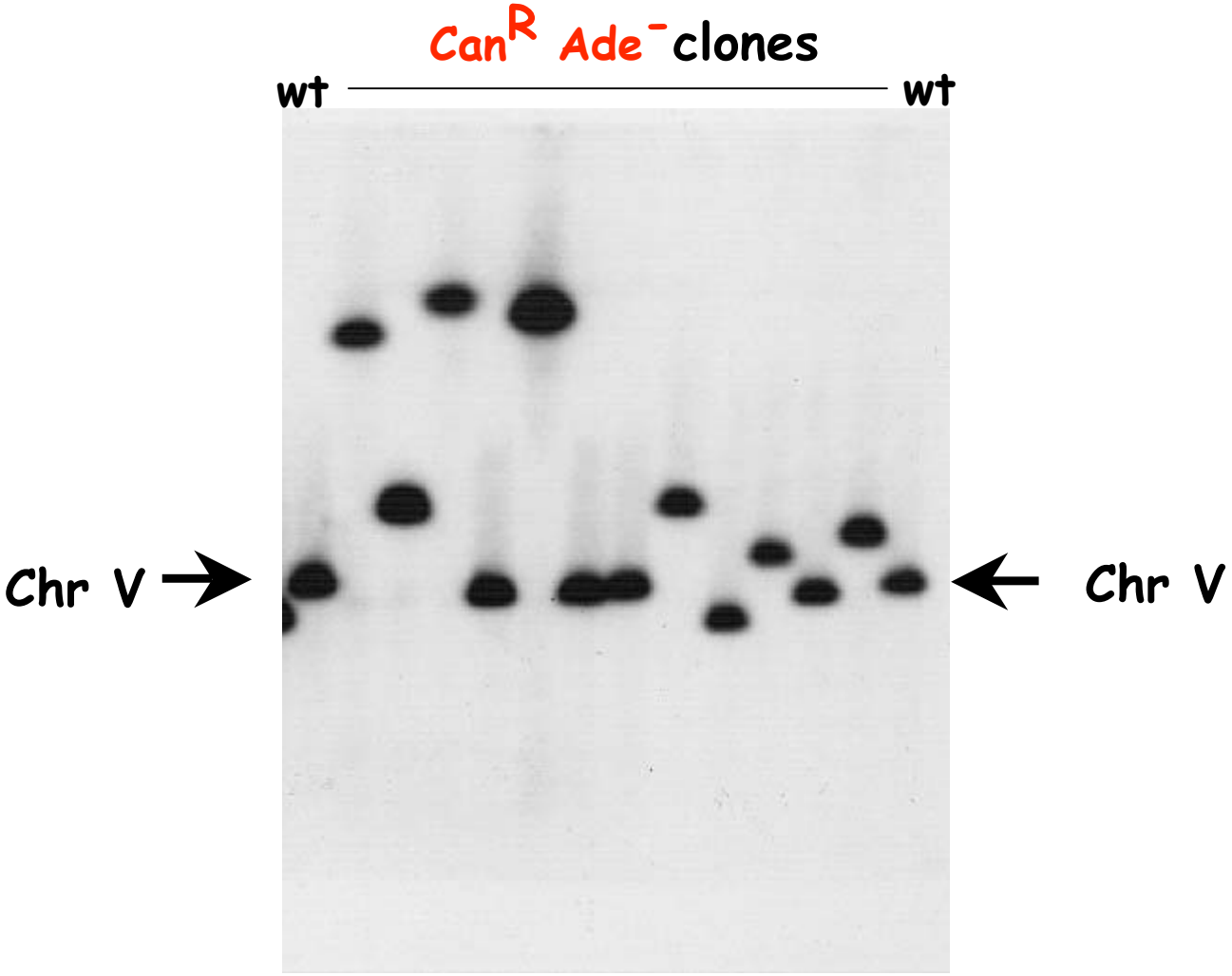
Working model for cruciform-mediated fragility



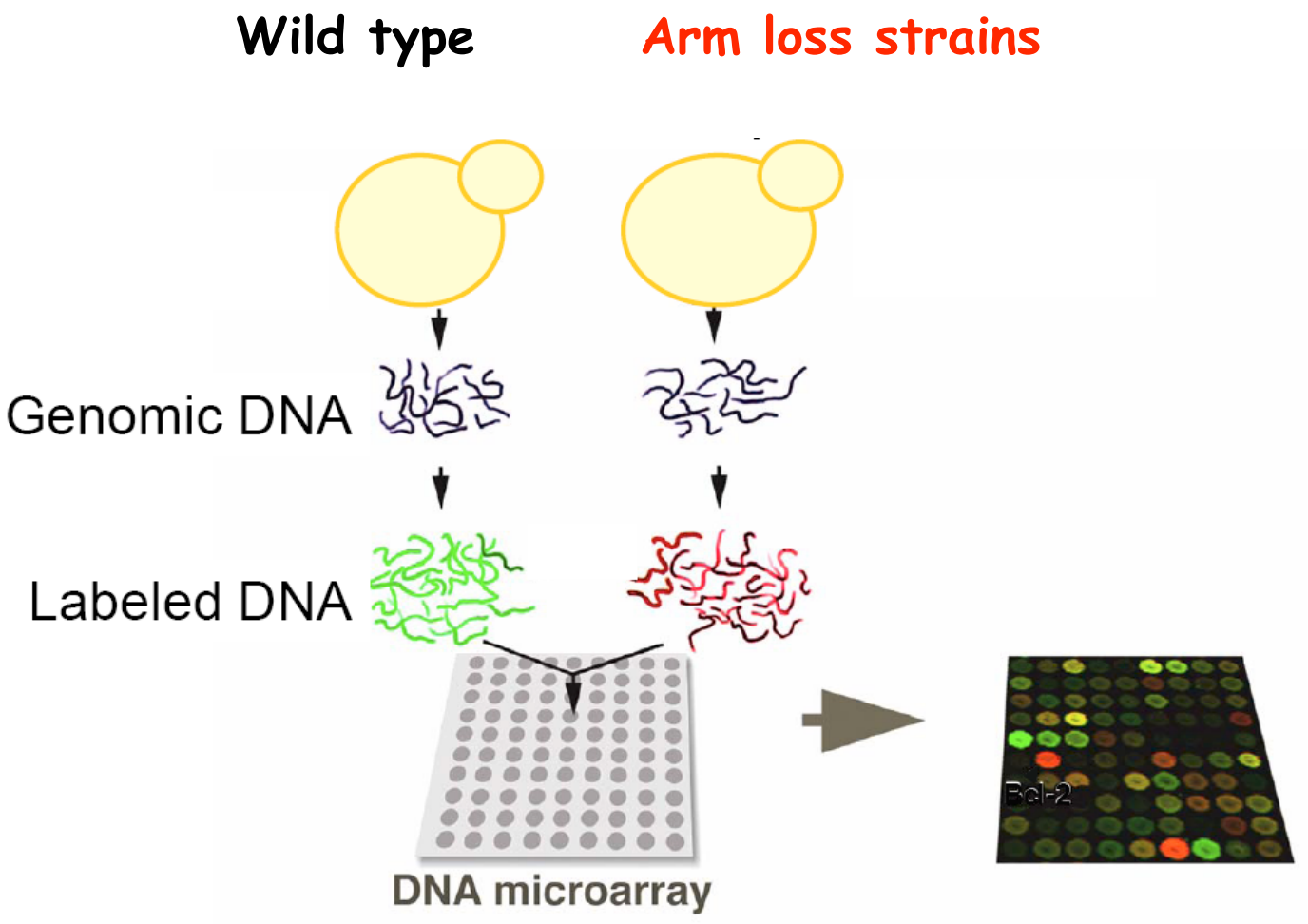
Working model for cruciform-mediated fragility



Inverted repeats stimulate specific pattern of GCR events

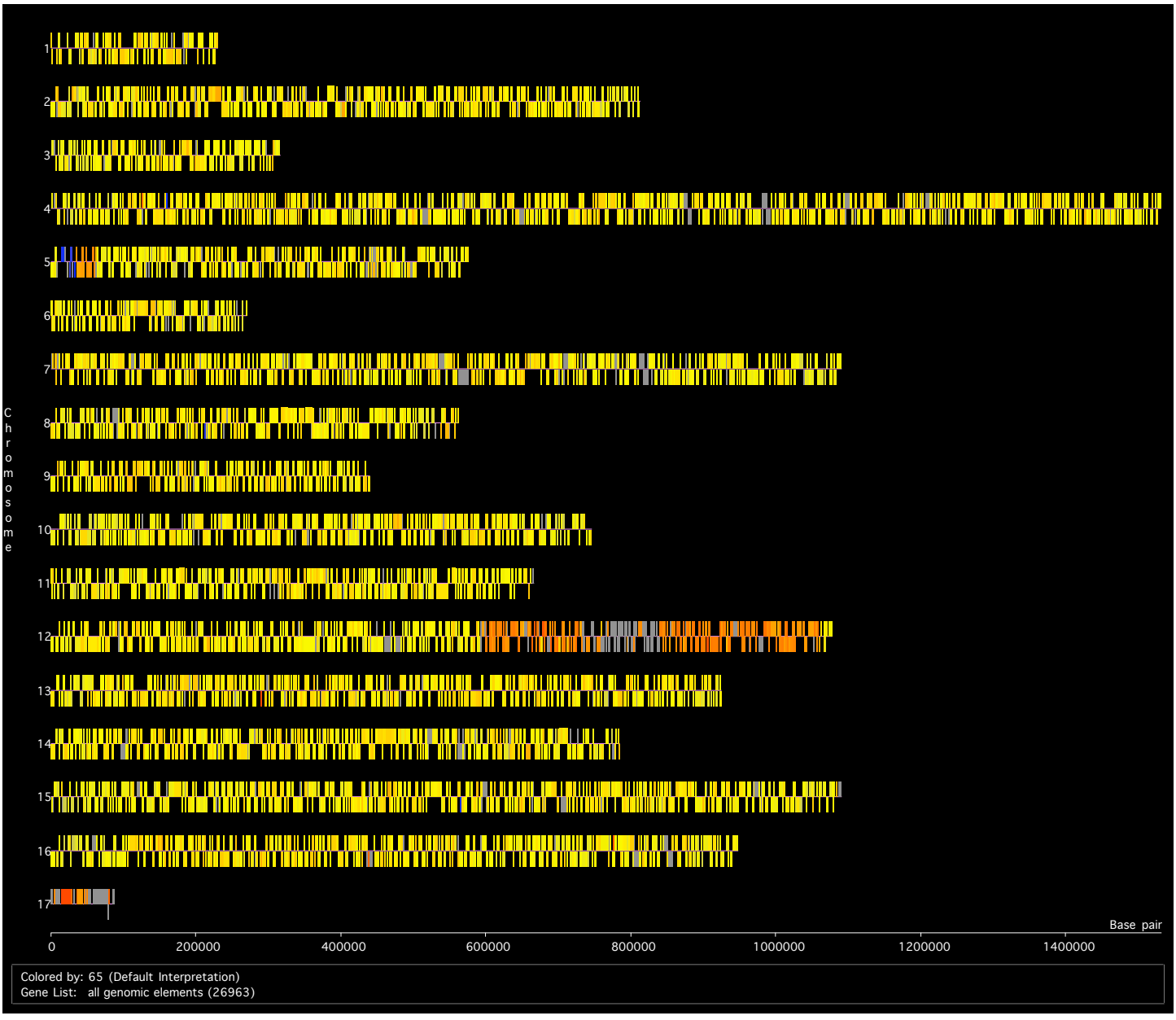


Comparative genome hybridization on microarrays



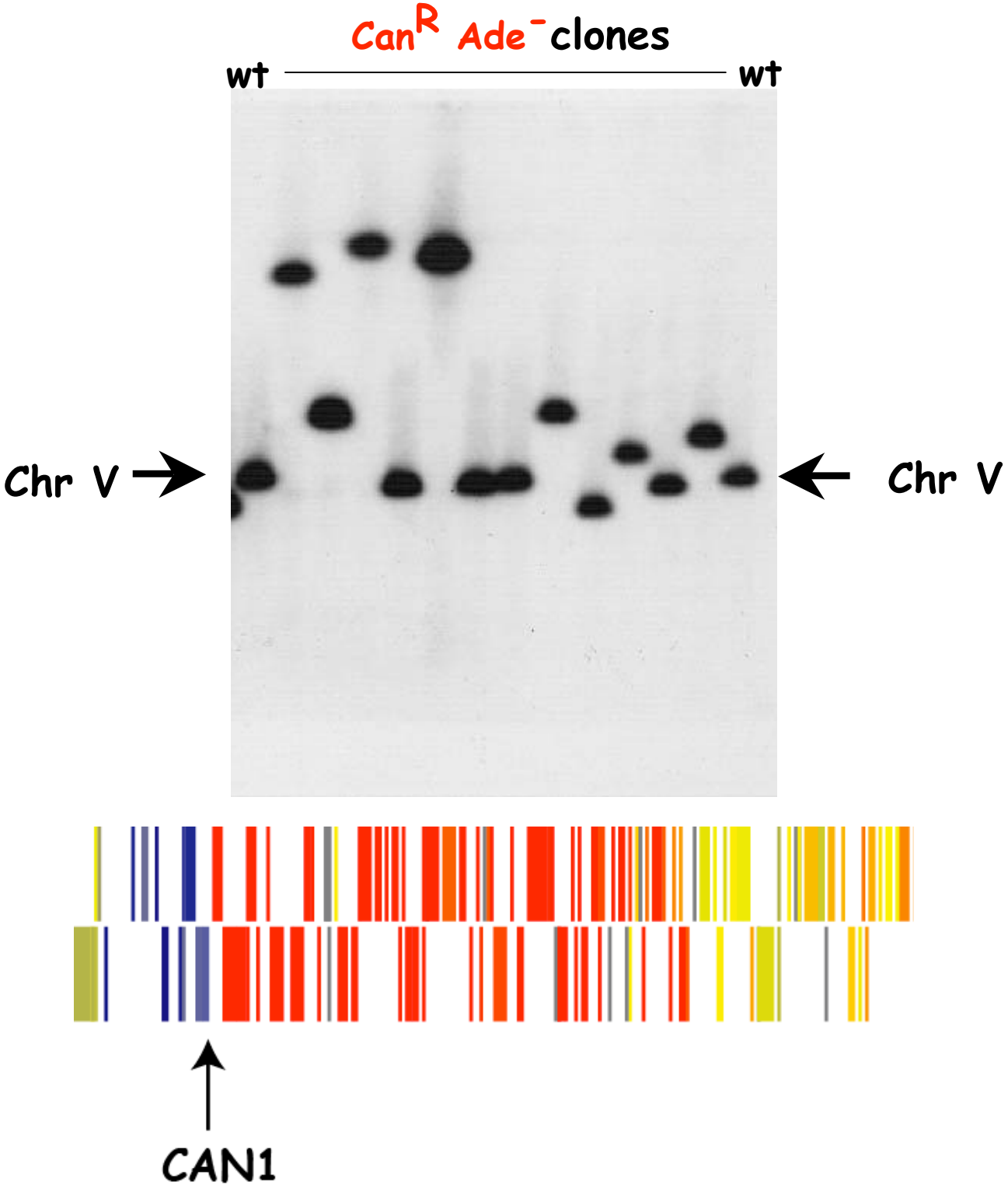
Collaboration with Dr. Thomas Petes' laboratory at Duke University

Analysis of genome architecture of Can^R Ade^- clones using CGH



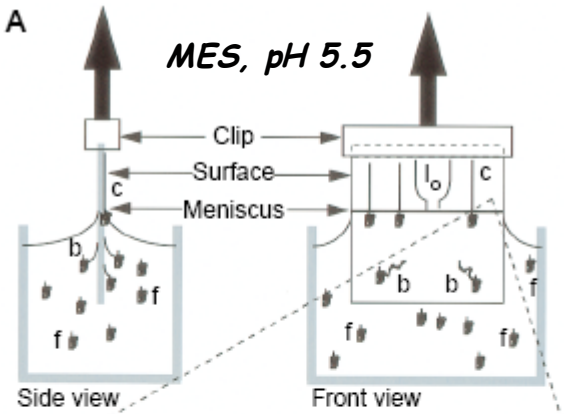
Narayanan et al, Cell 2006

Inverted repeats stimulate specific pattern of GCR events

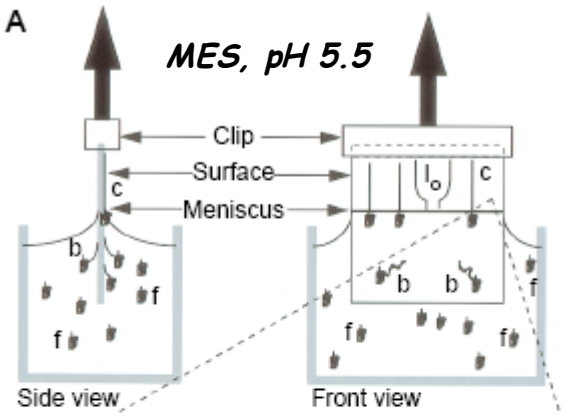


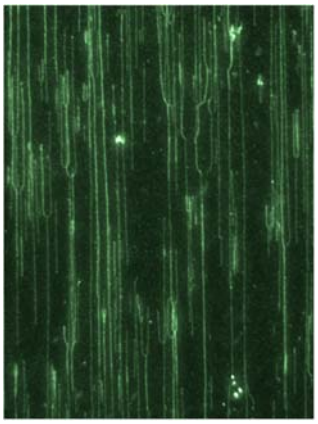
Collaboration with Piotr Mieczkowski and Tom Petes
Duke University

Dynamic Molecular Combing and FISH to analyze the structure of rearranged regions



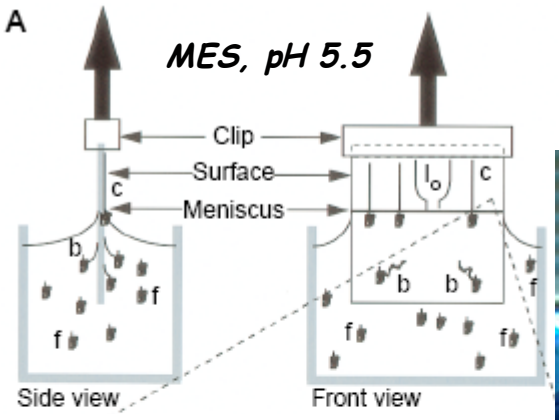
Dynamic Molecular Combing and FISH to analyze the structure of rearranged regions

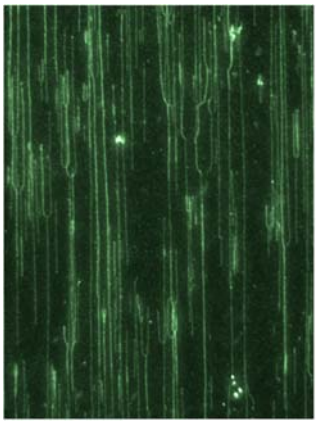


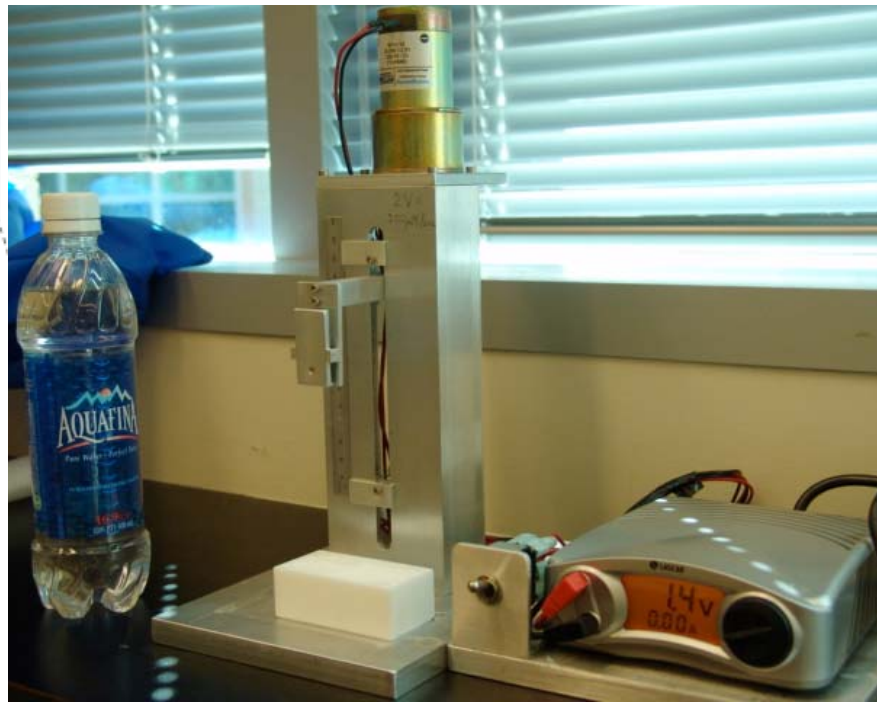


Michalet et al, Science 1997 (A. Bensimon lab, Pasteur Institute)

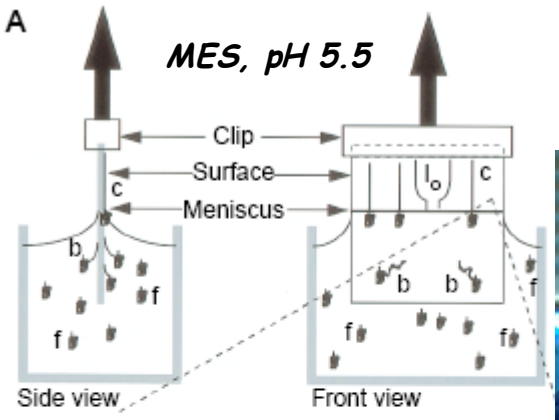
Dynamic Molecular Combing and FISH to analyze the structure of rearranged regions

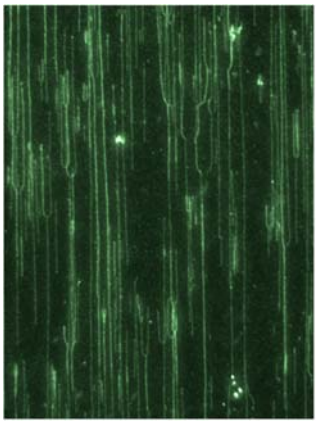


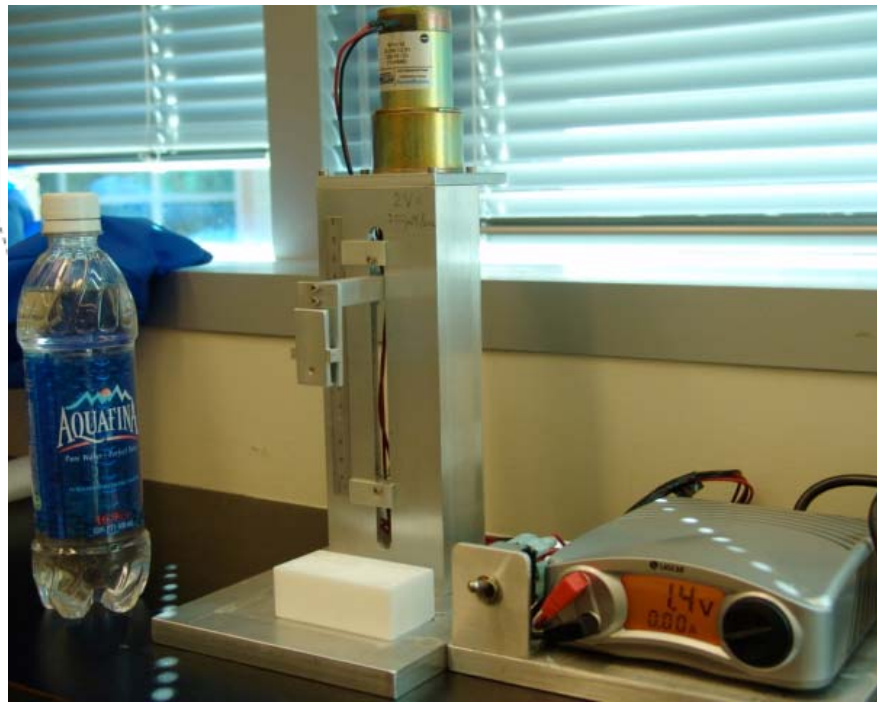




Extrachromosomal amplicon is linear inverted dimer







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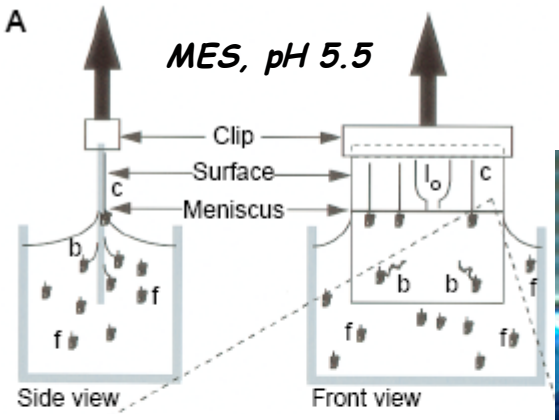
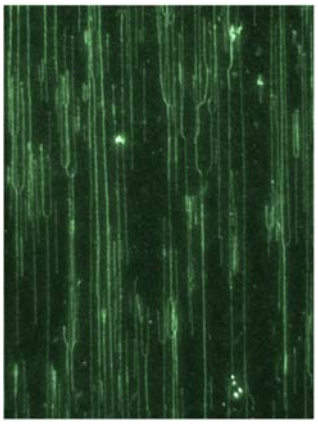
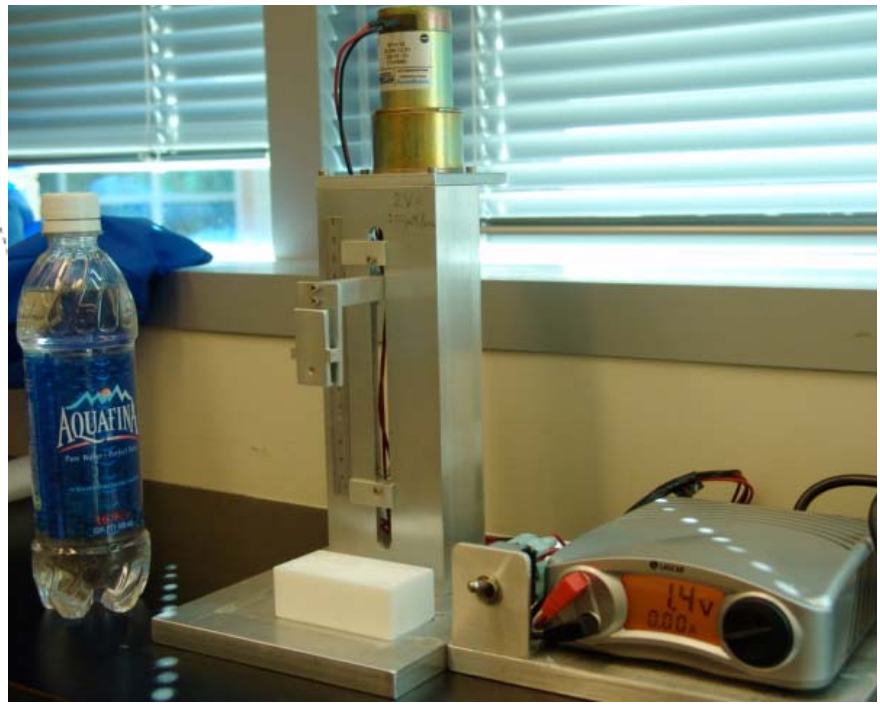
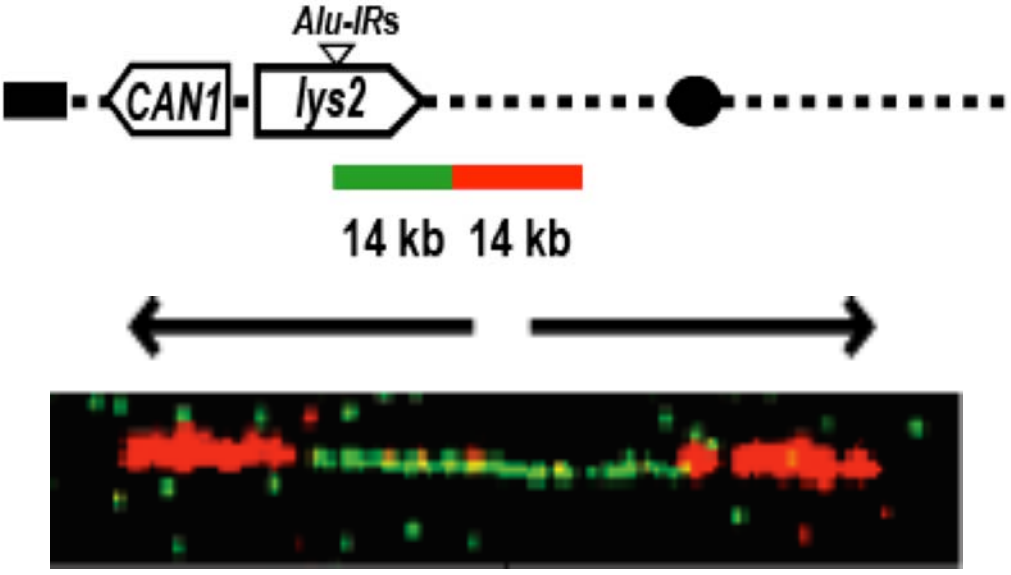
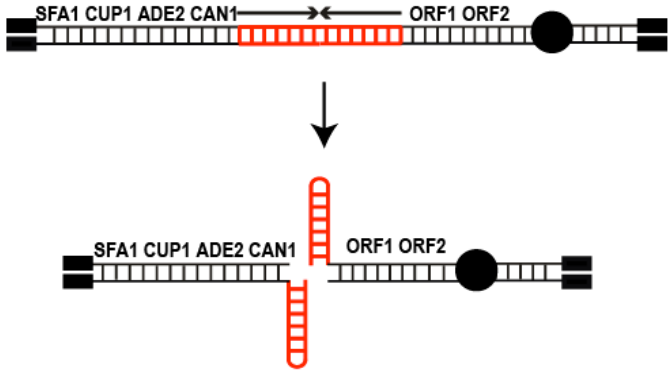








Mechanism of gross chromosomal rearrangements induced by inverted *Alu* repeats



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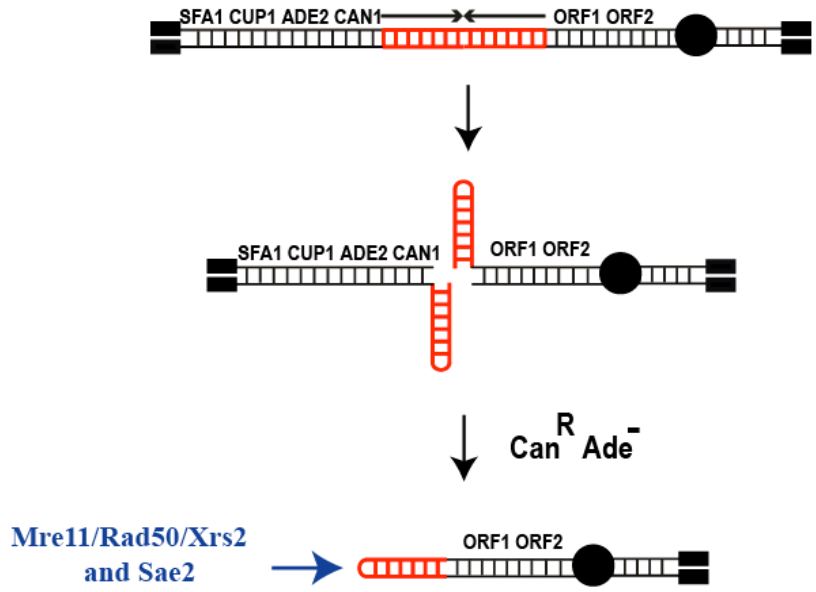

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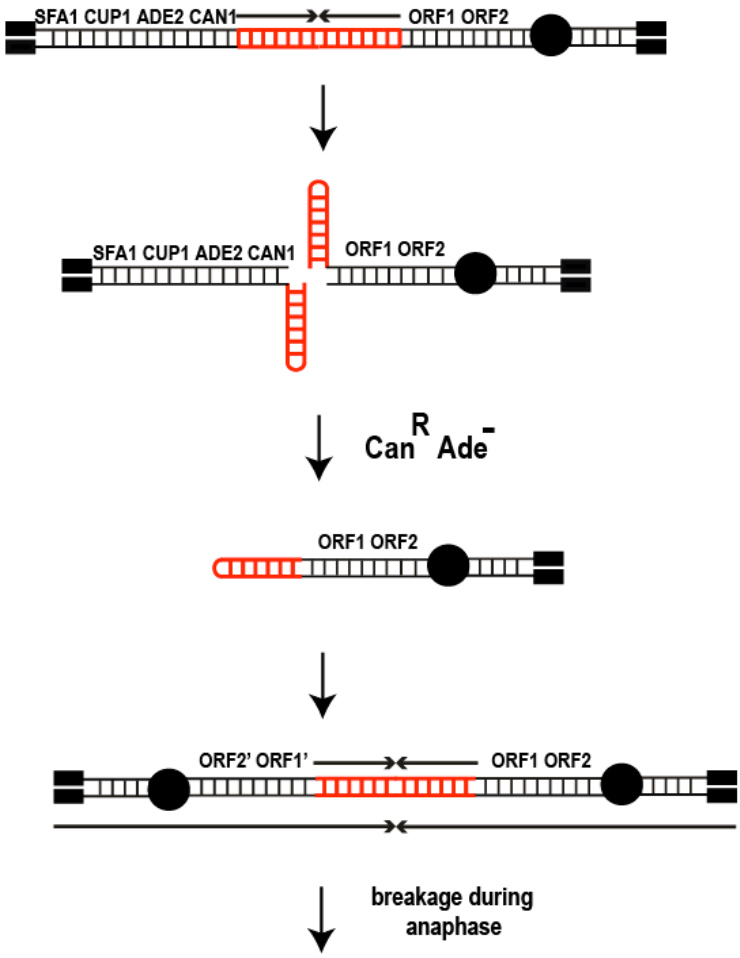

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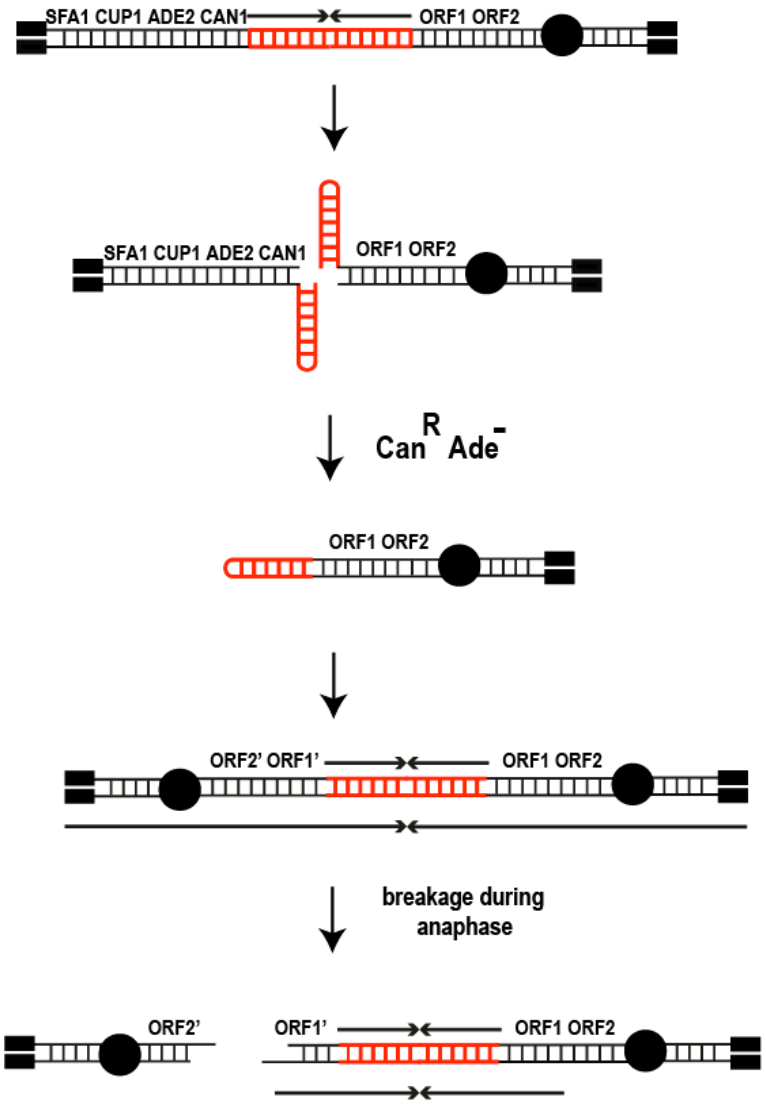

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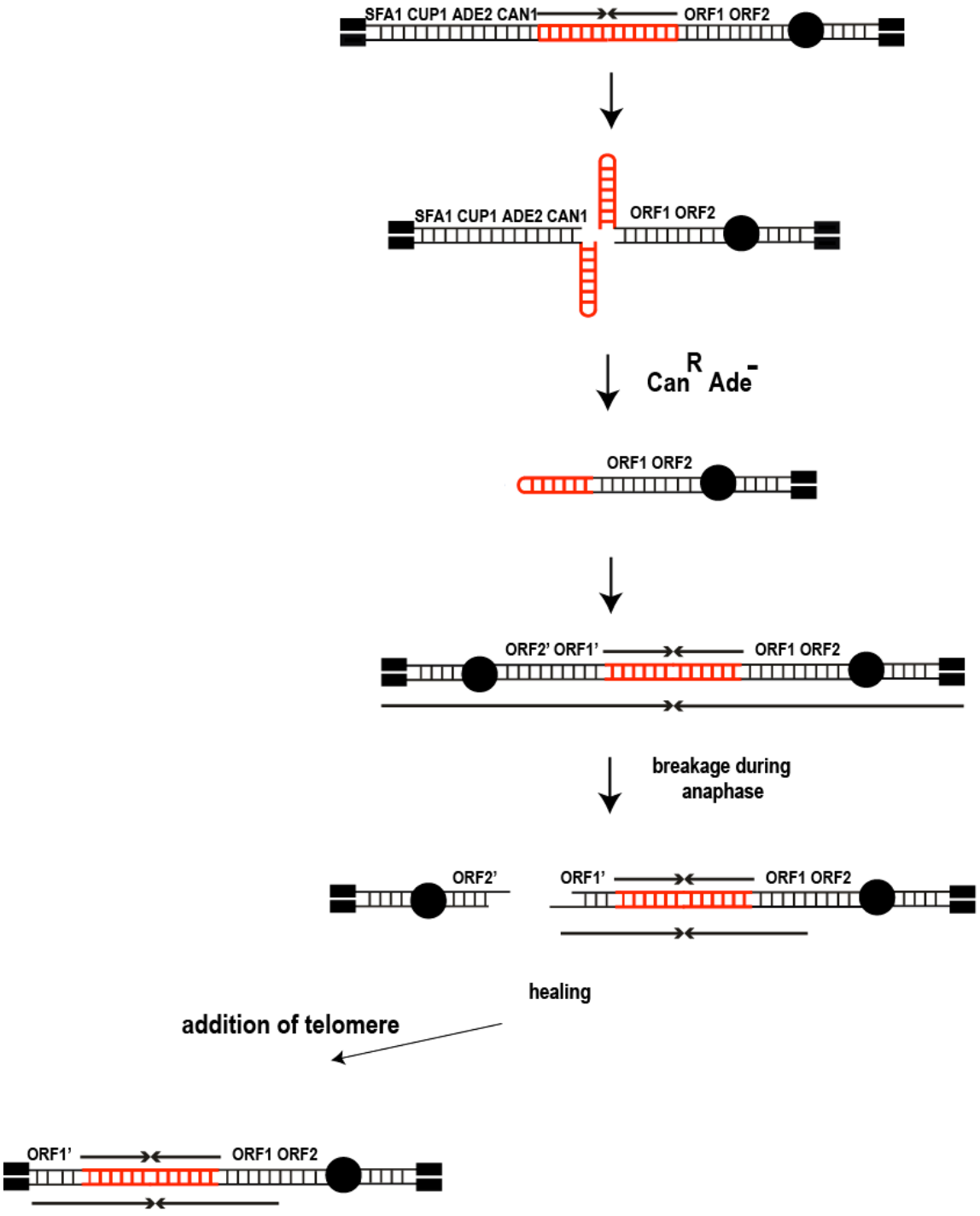
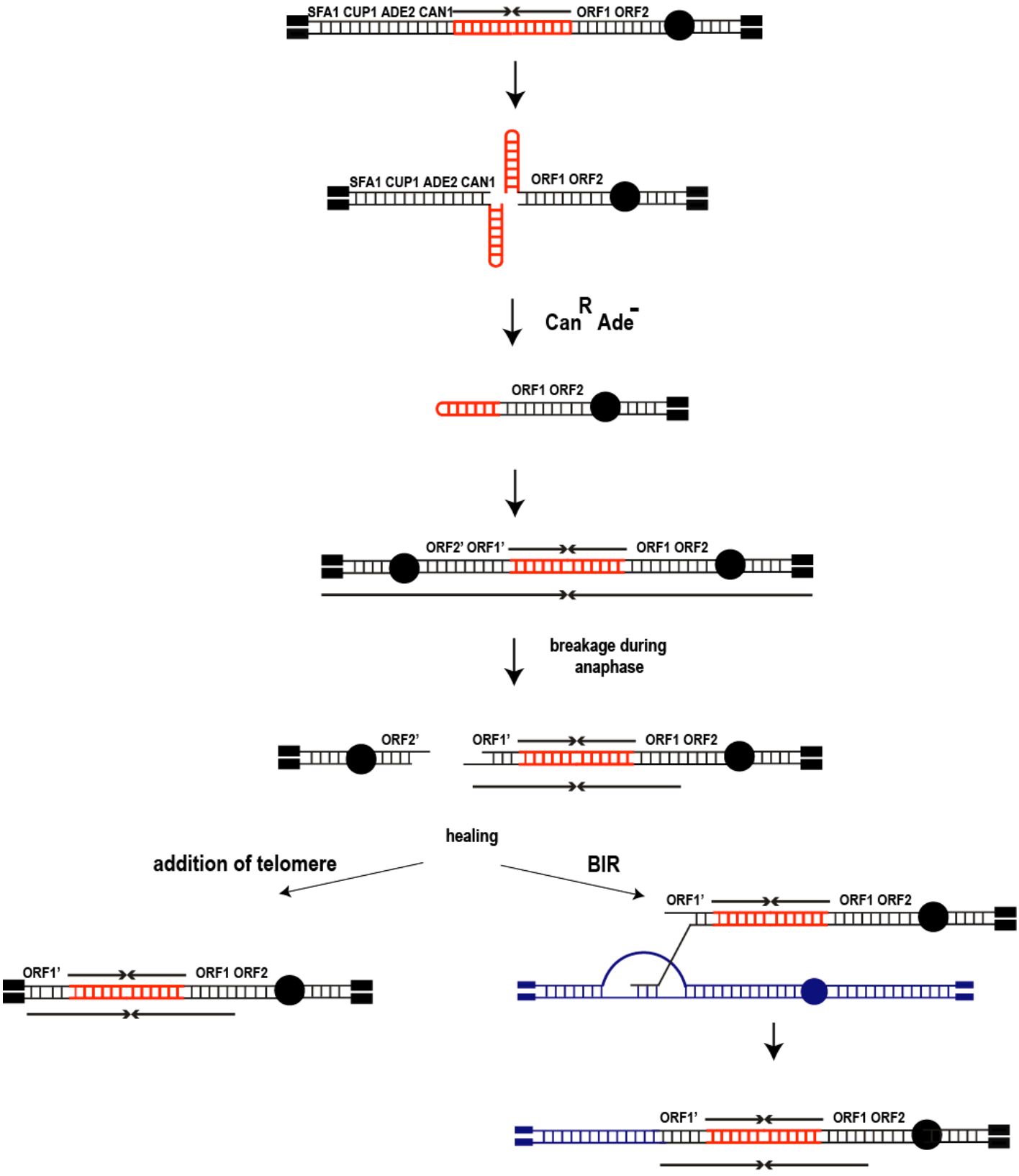
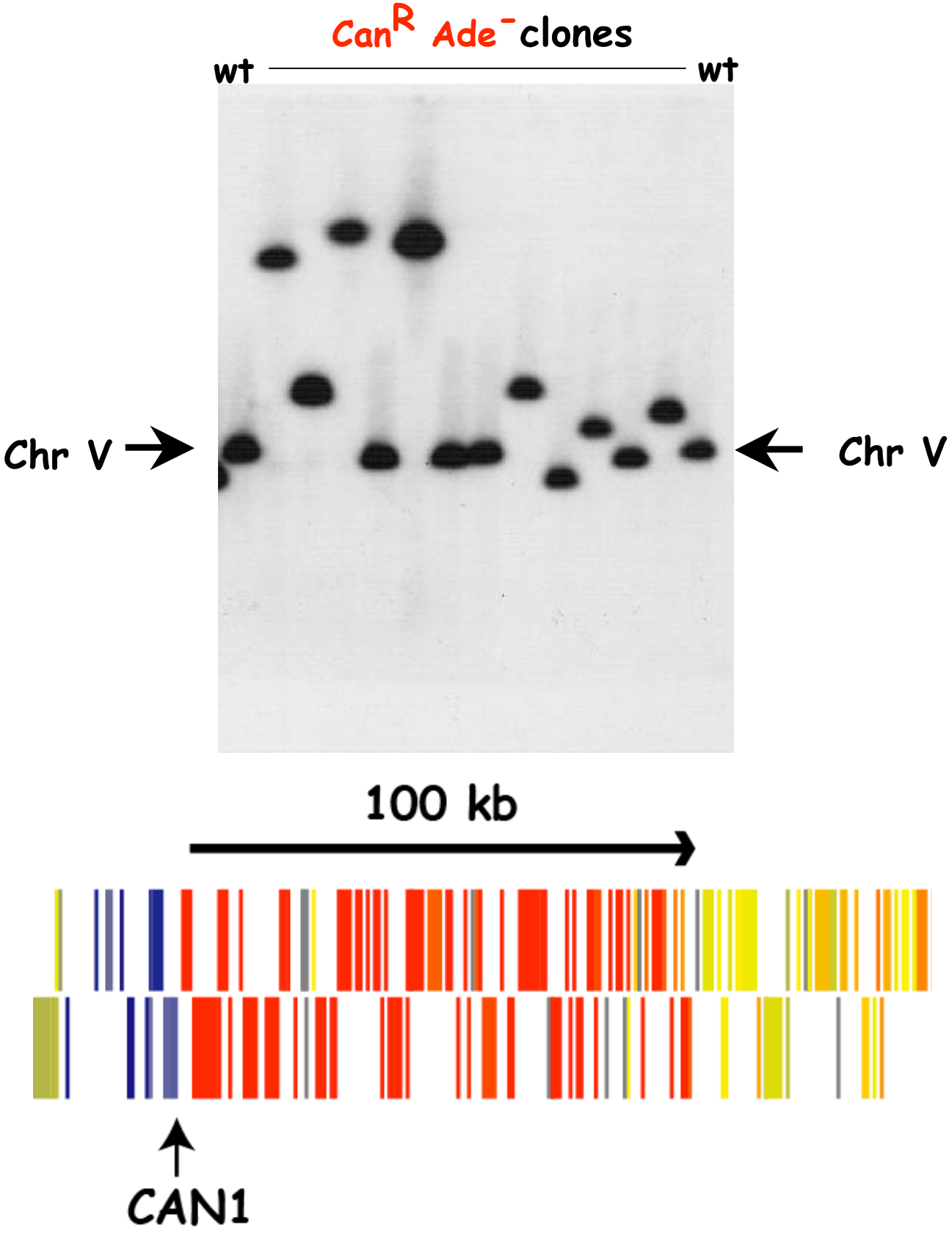


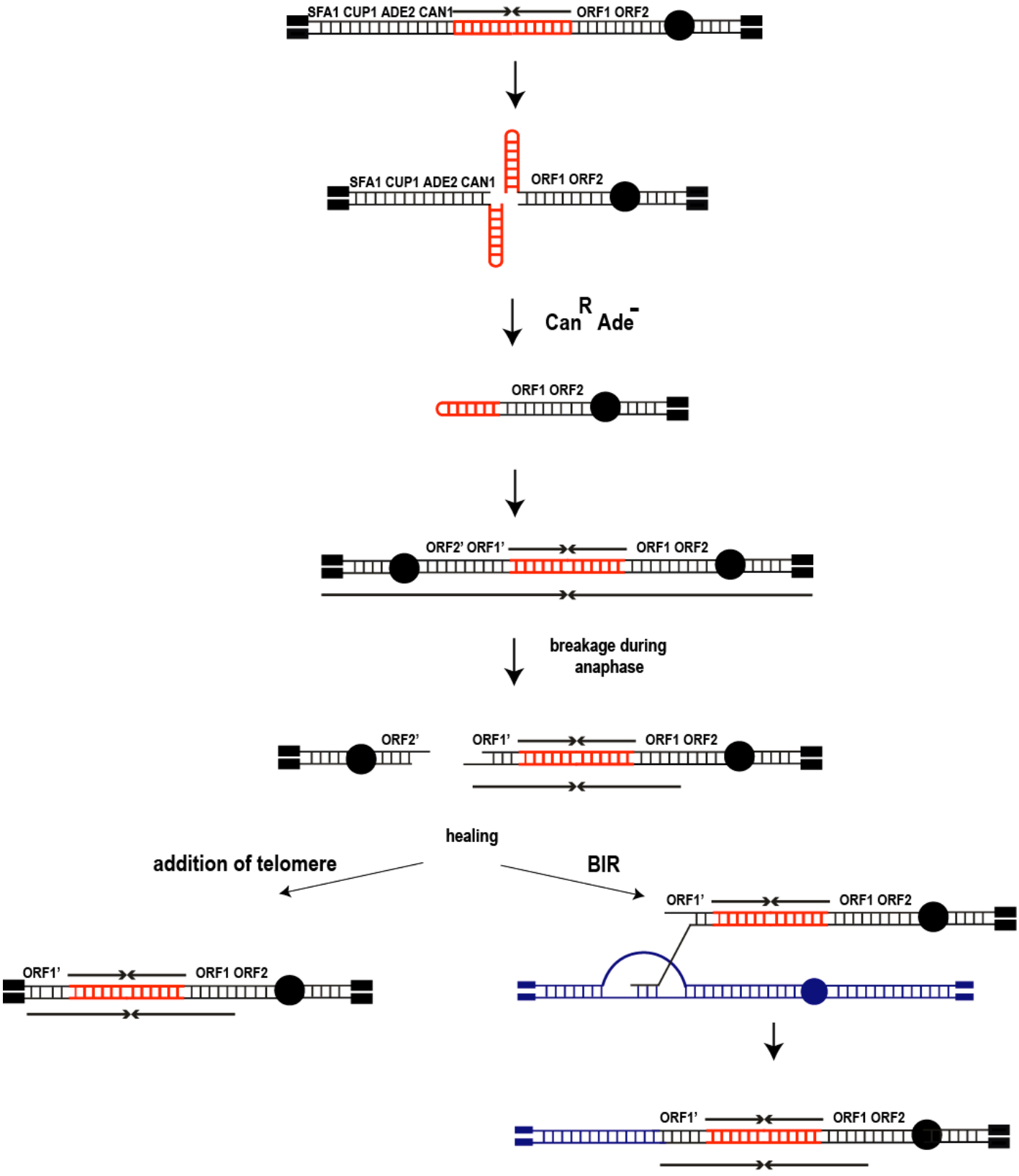
Mechanism of gross chromosomal rearrangements induced by inverted *Alu* repeats



Inverted repeats stimulate specific pattern of GCR events

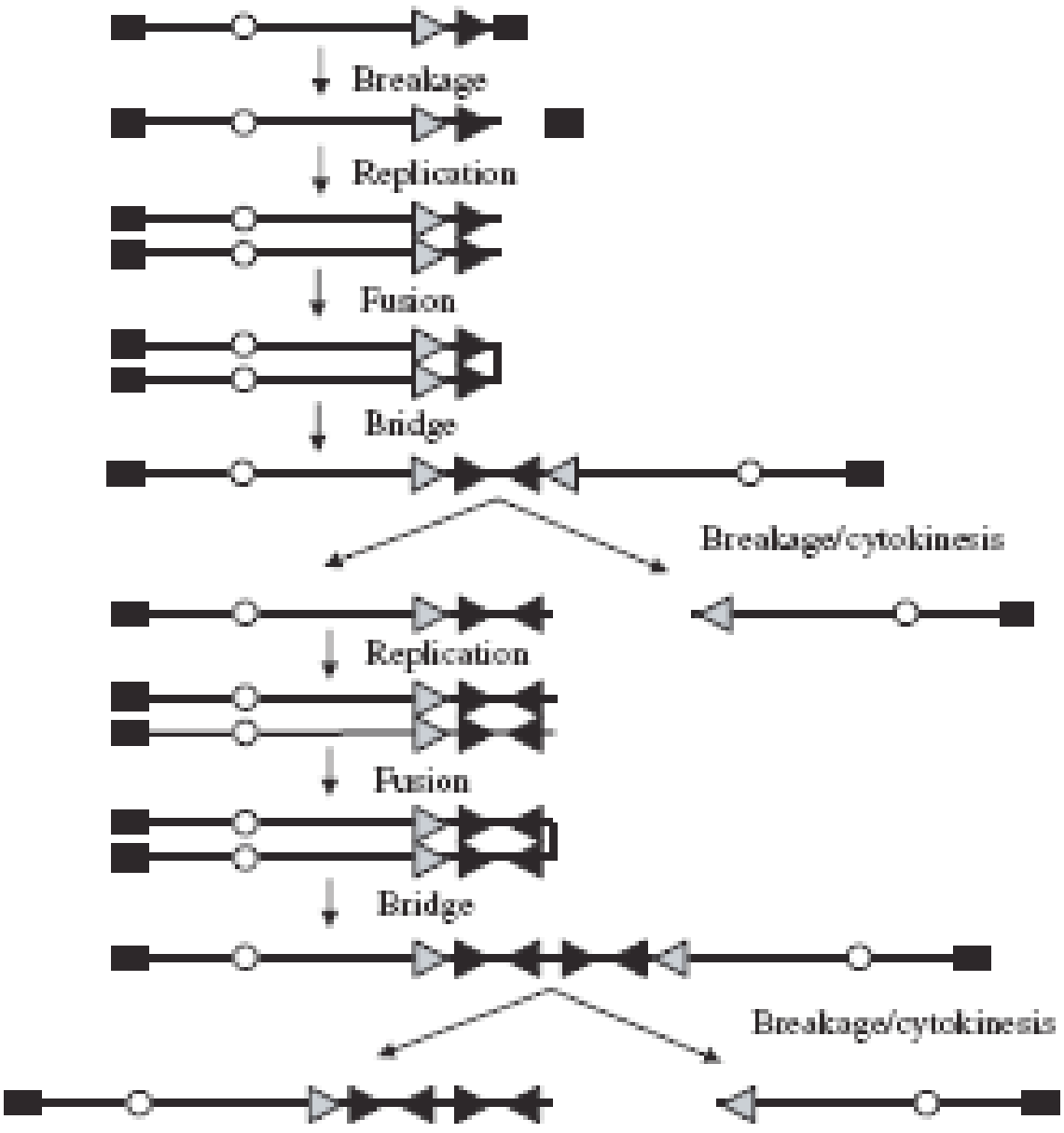


Mechanism of gross chromosomal rearrangements induced by inverted *Alu* repeats

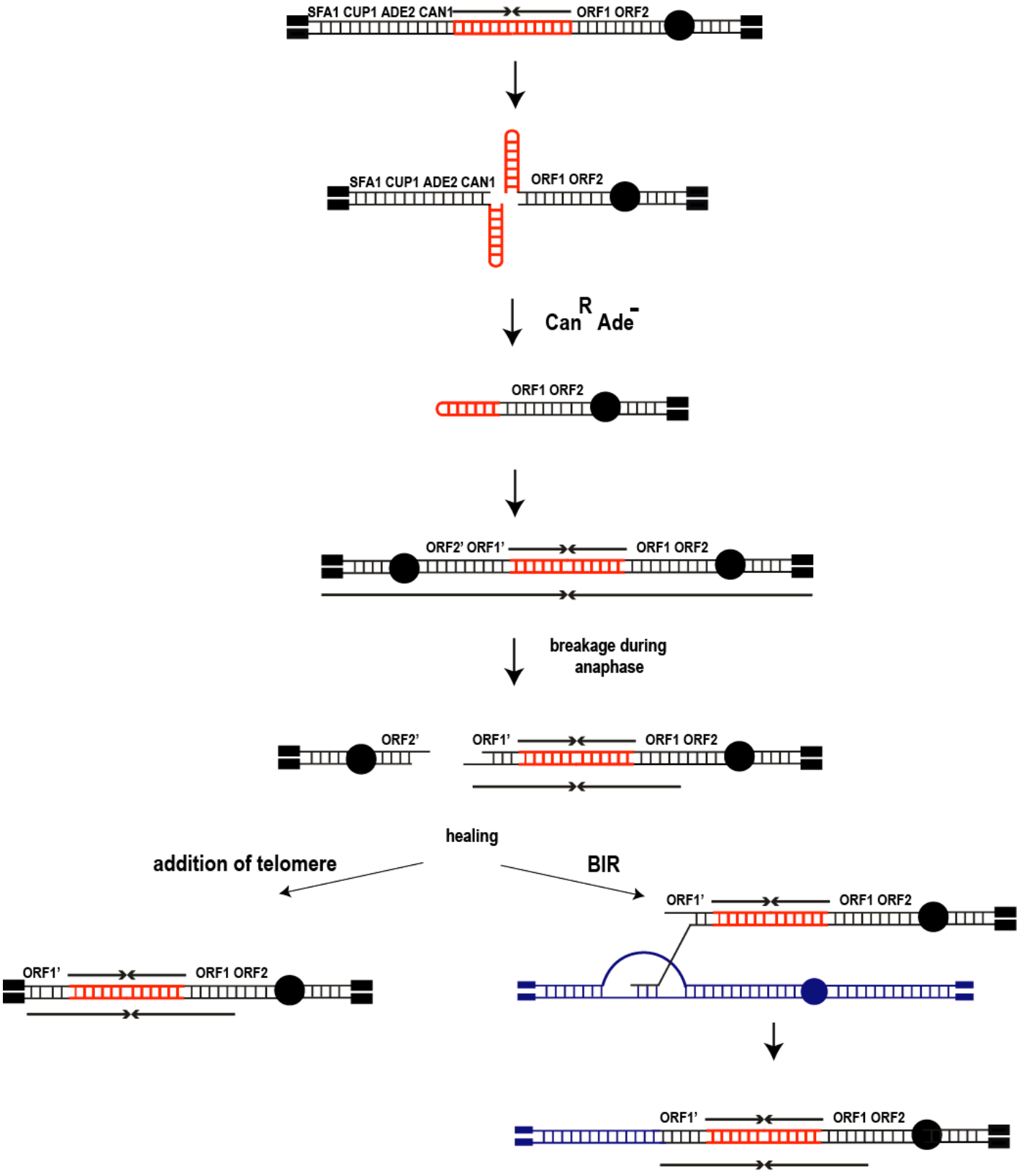


Breakage/fusion/bridge cycle as a mechanism for gene amplification

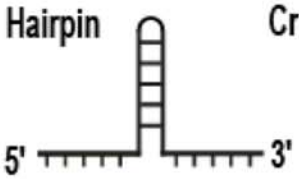
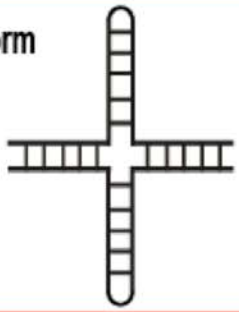
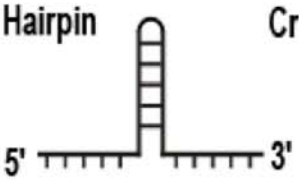
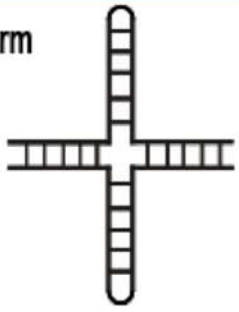
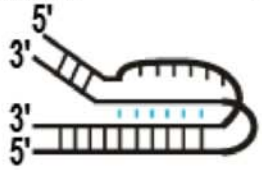
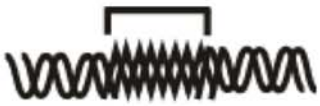
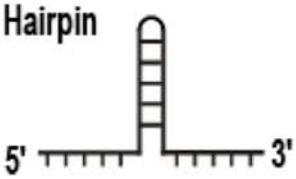
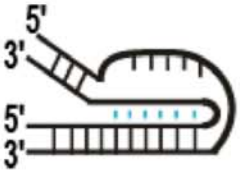
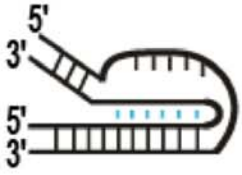
1941



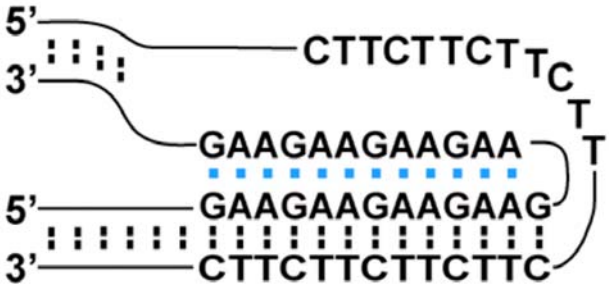
Mechanism of gross chromosomal rearrangements induced by inverted *Alu* repeats



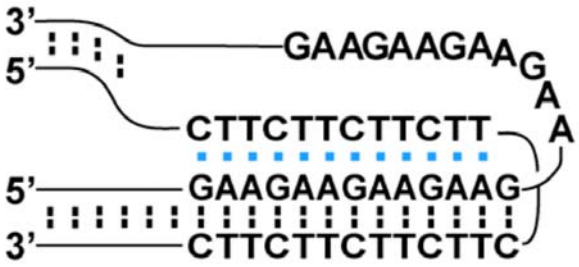
At-risk motifs

At-risk motif	Alternative structure
Inverted repeats	Hairpin  Cruciform 
Dinucleotide repeats (CG)n (AT)n (GA)n (TG)n	Hairpin  Cruciform  H-DNA  Z-DNA 
Trinucleotide repeats (CGG/CCG)n (CTG/CAG)n (GAA/TTC)n	Hairpin  H-DNA 
Pu-Py mirror repeats	H-DNA 

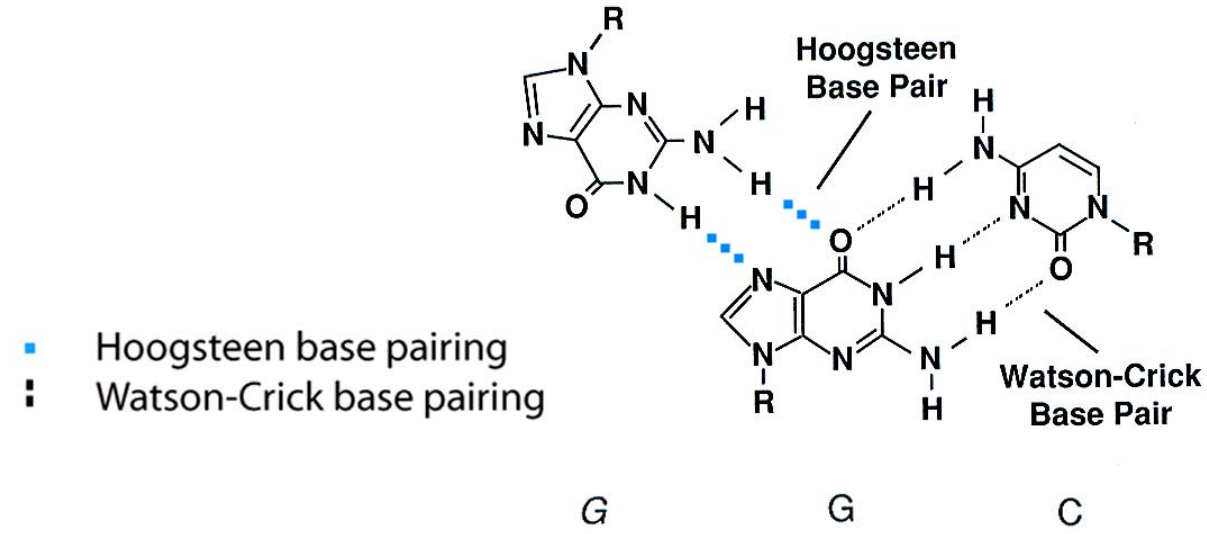
GAA/TTC repeats can adopt triplex (H-) DNA



Pu **Pu Py (Hu3)**

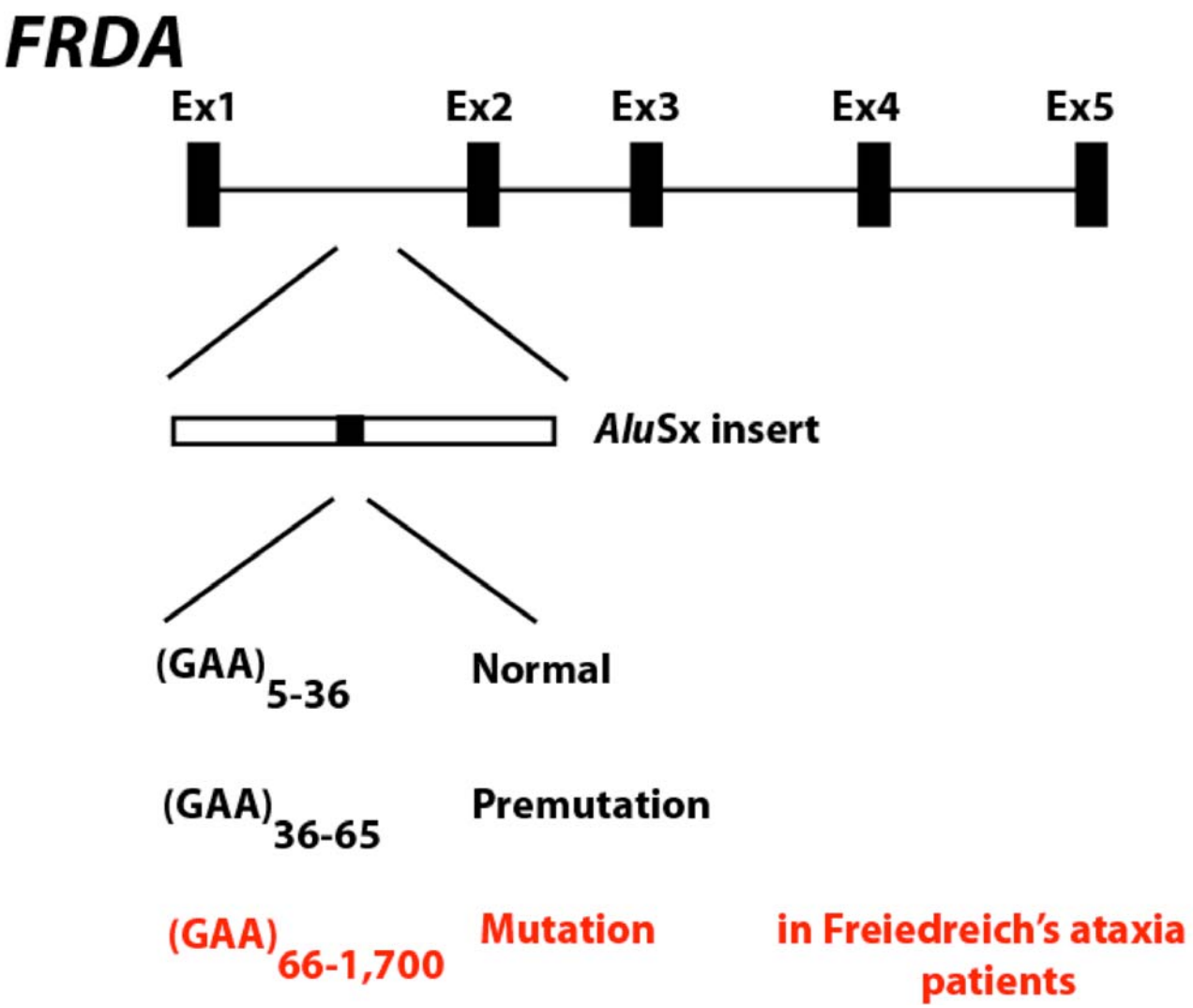


Py **Pu Py (Hy5)**

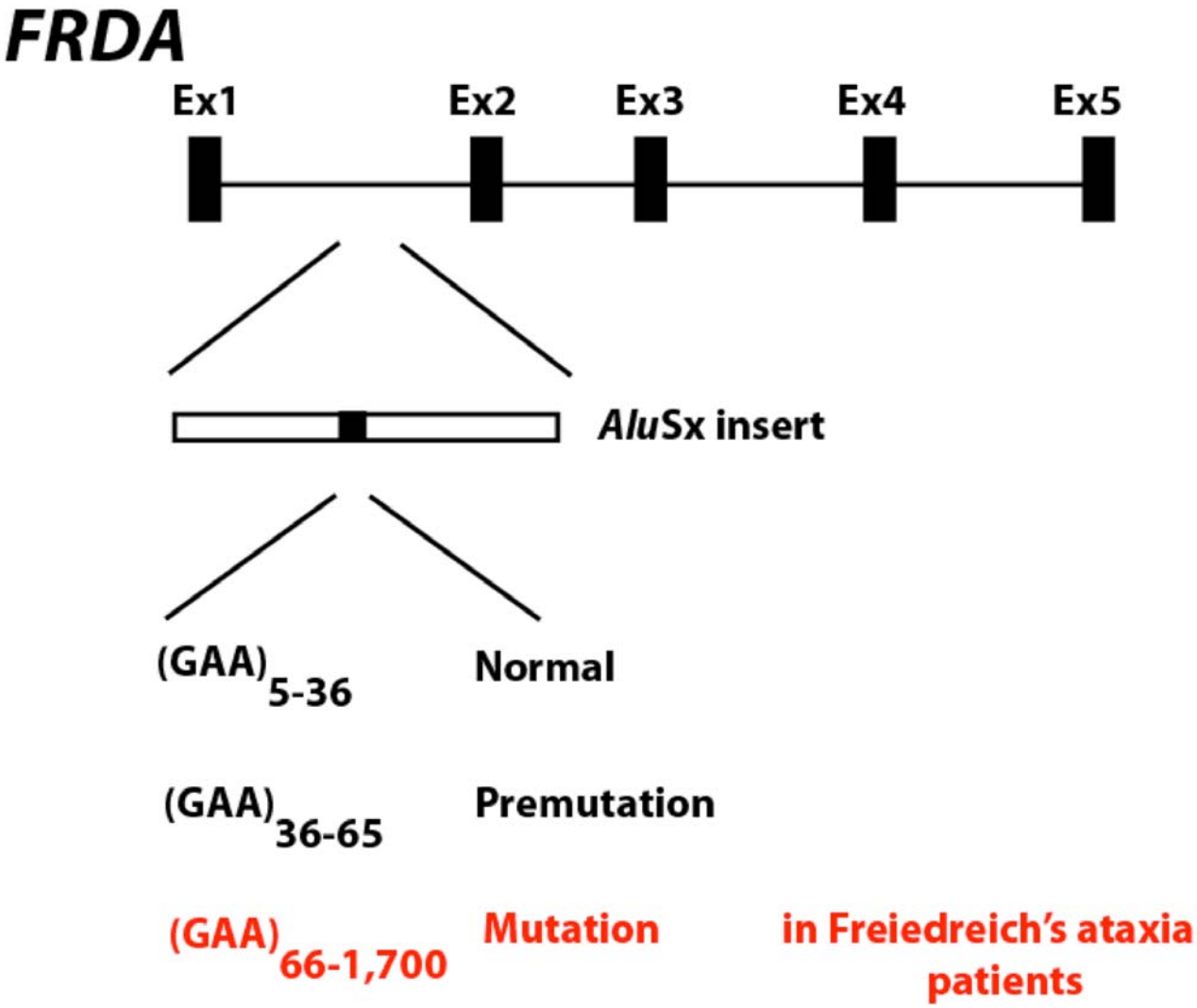


Mirkin et al., Nature 1987

GAA/TTC expansions in the human genome

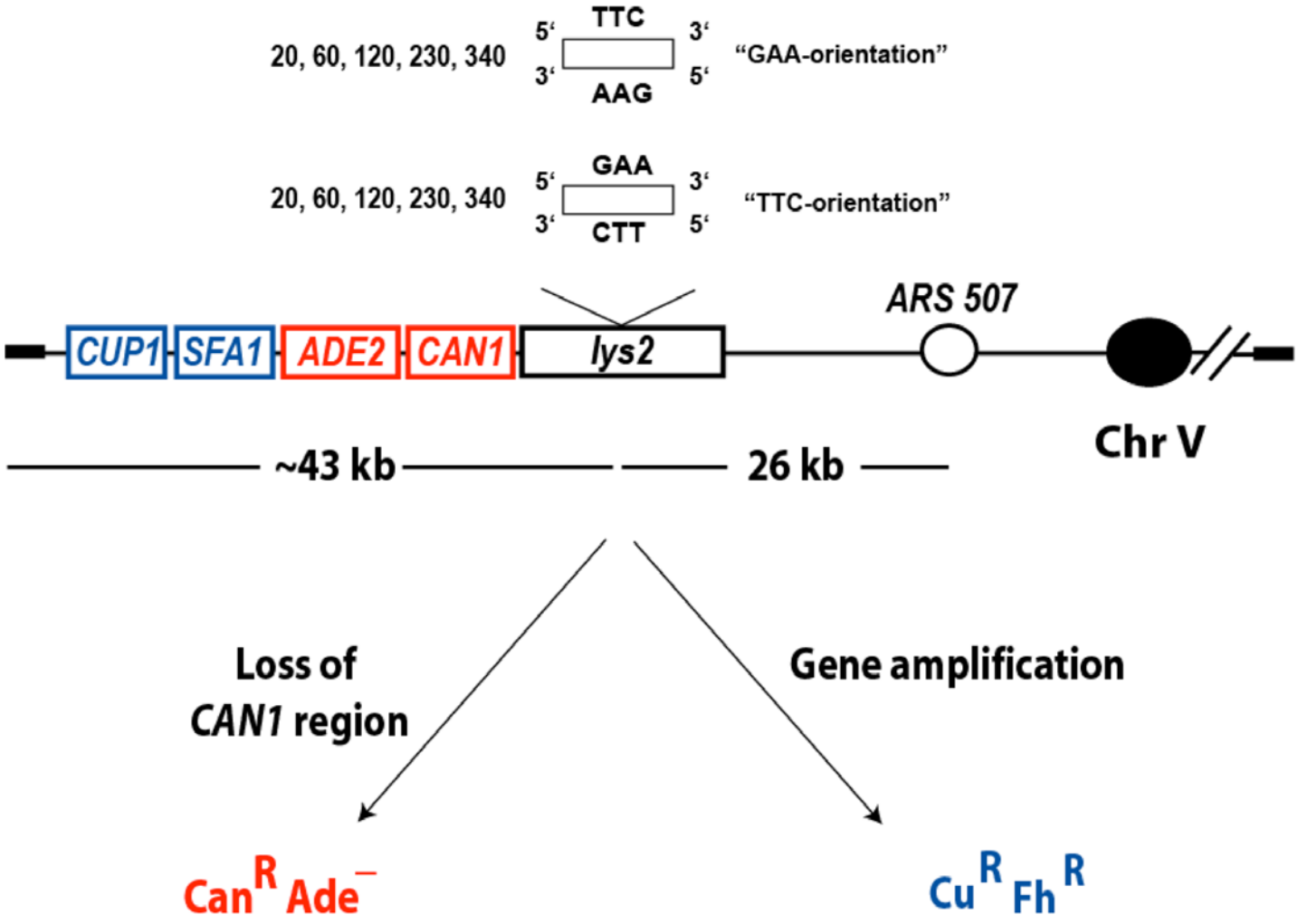


GAA/TTC expansions in the human genome



There are nearly a 1000 other loci that have GAA repeats!

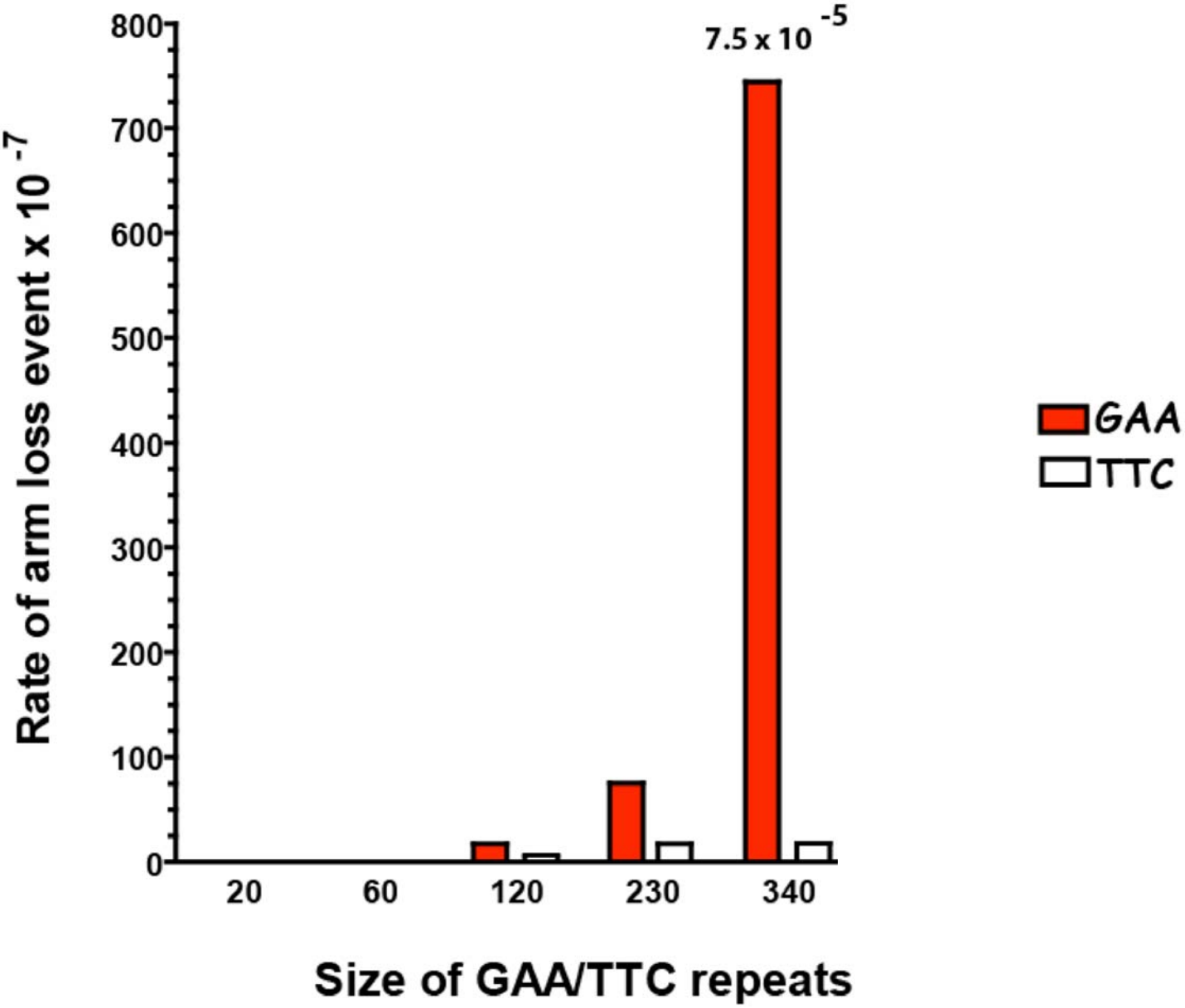
Can long GAA/TTC tracks induce chromosomal instability?



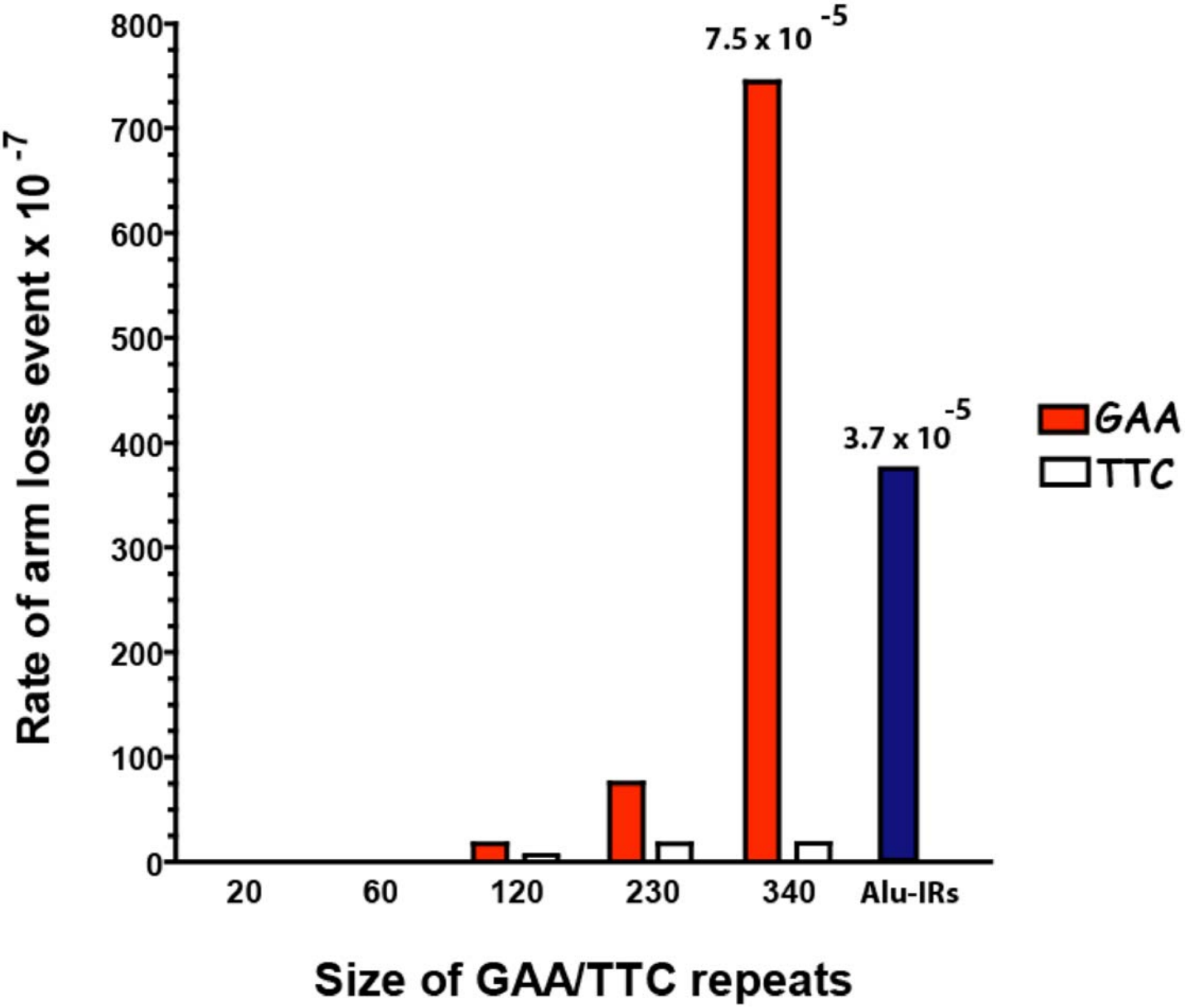
Friedreich's ataxia patients have 66-1,700 repeats!

There are nearly a 1000 other loci in the human genome that contain GAA/TTC tracks!

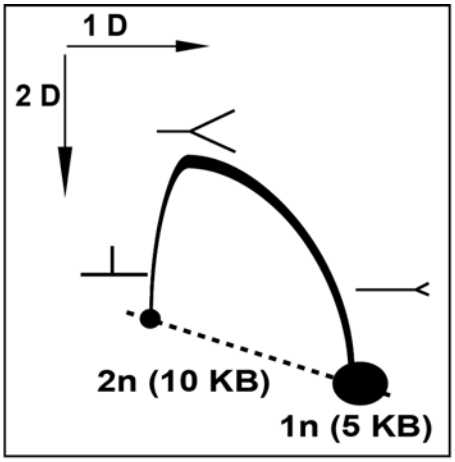
Induction of arm loss events depends on size and orientation of GAA/TTC repeats

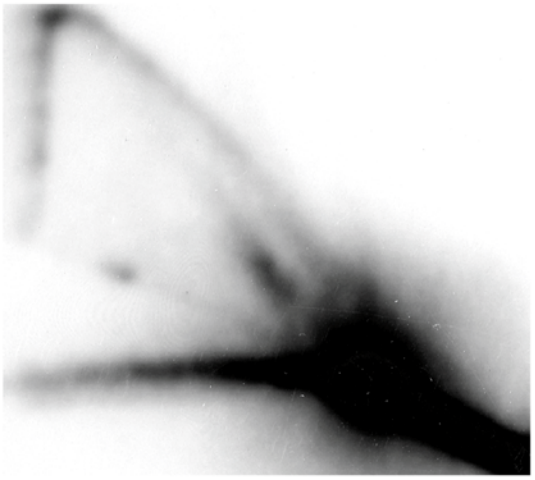


Induction of arm loss events depends on size and orientation of GAA/TTC repeats

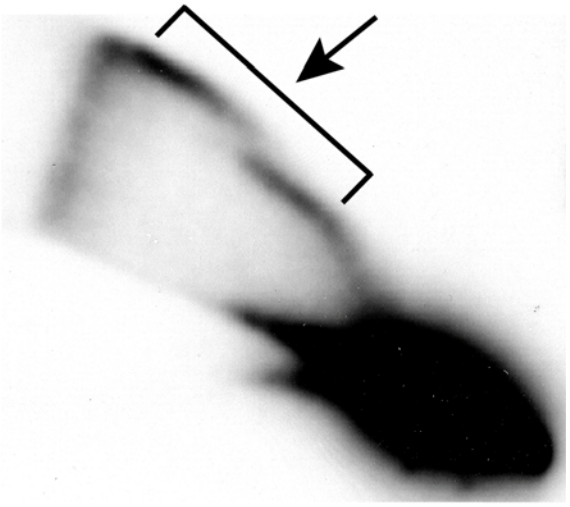


340 copies of GAA/TTC repeats inhibit progression of chromosomal DNA replication





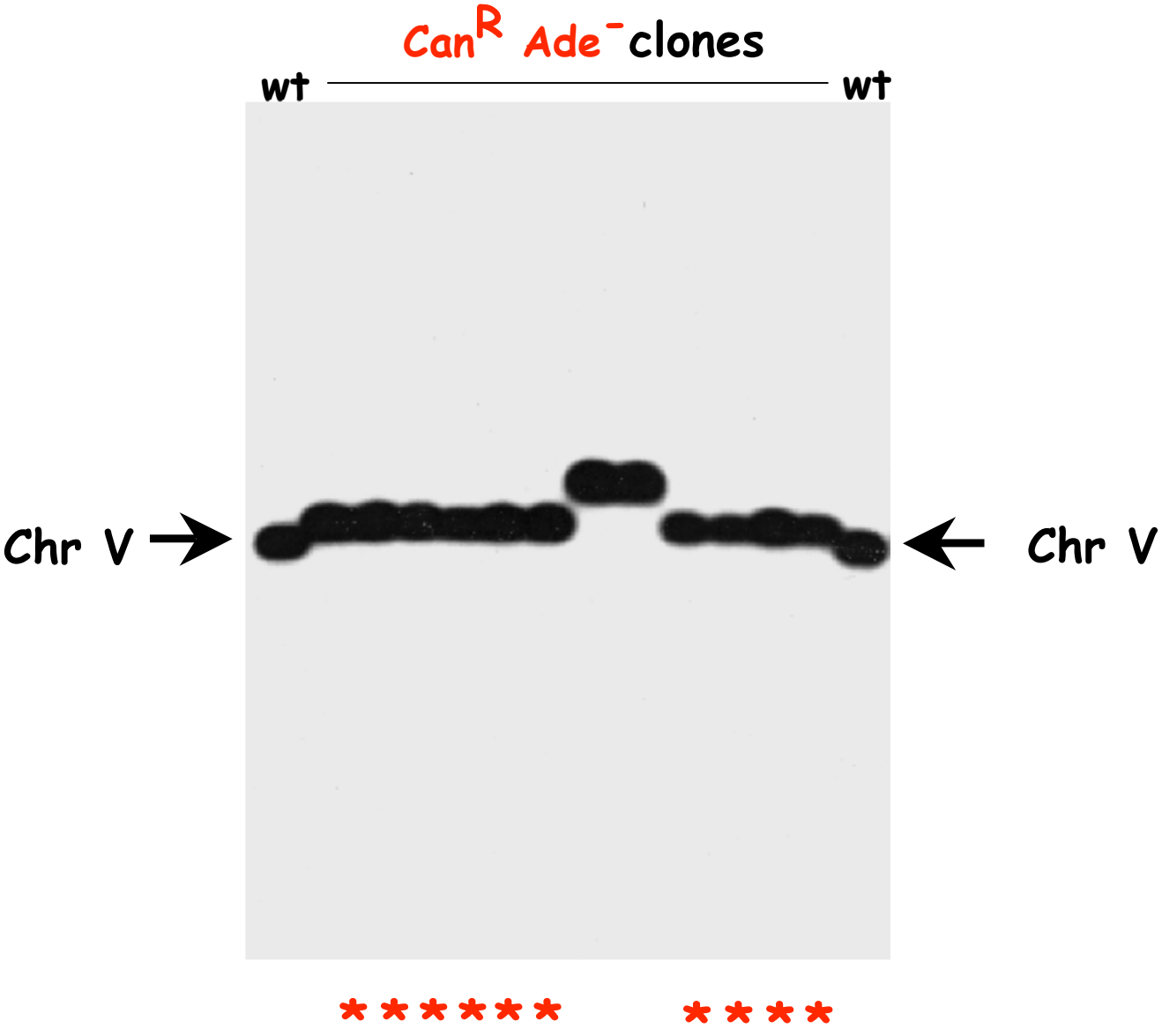
$(TTC)_{340}$



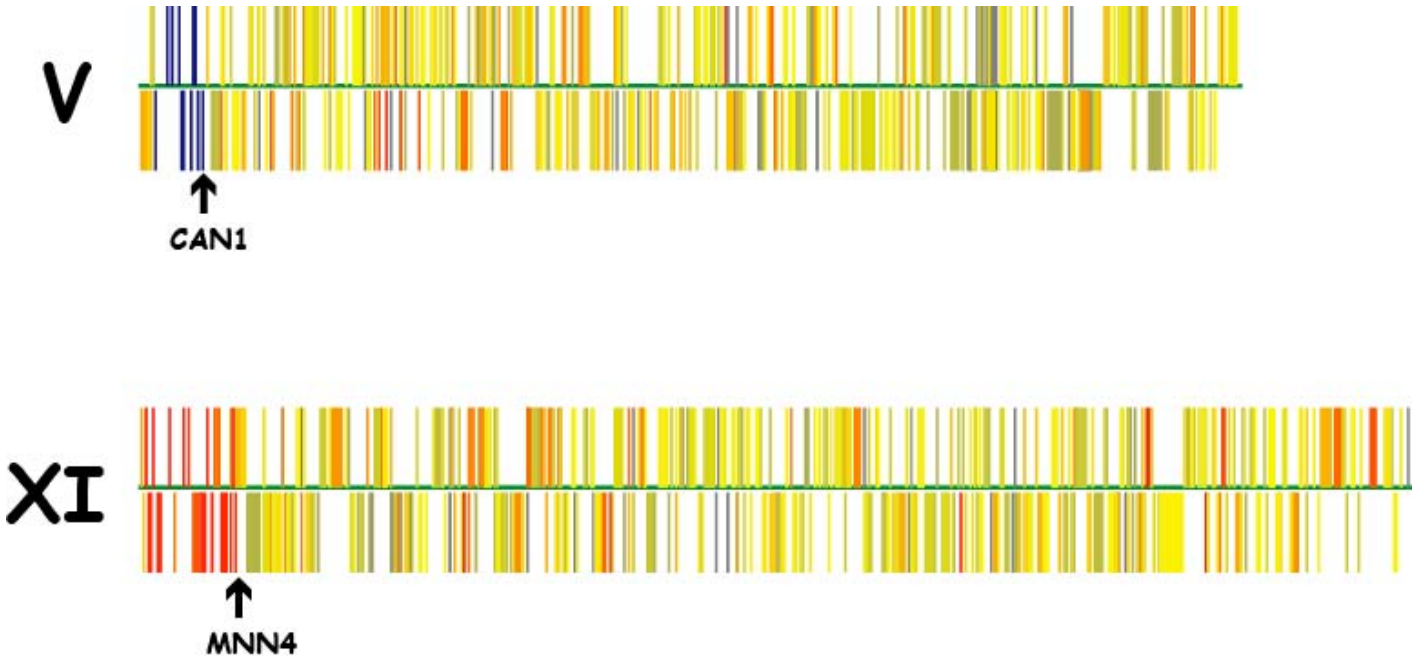
$(GAA)_{340}$

only in one orientation!

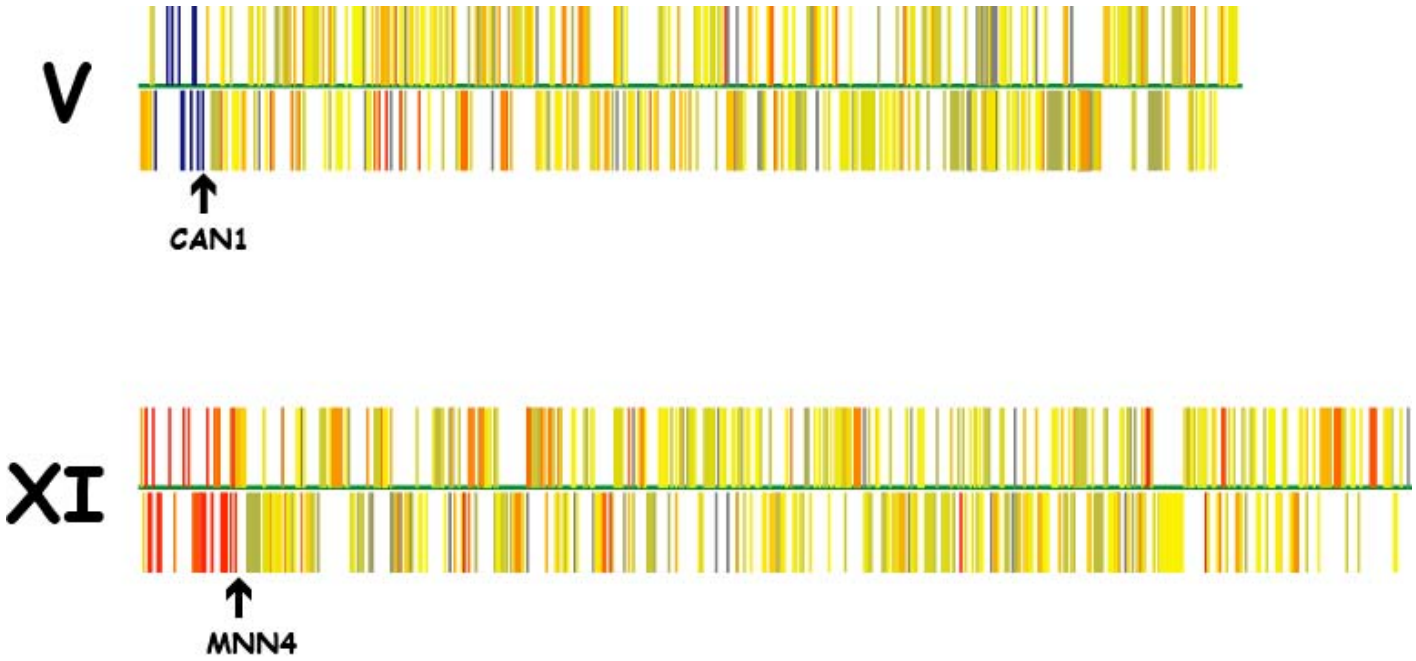
GAA/TTC repeats induce specific type of chromosomal rearrangements

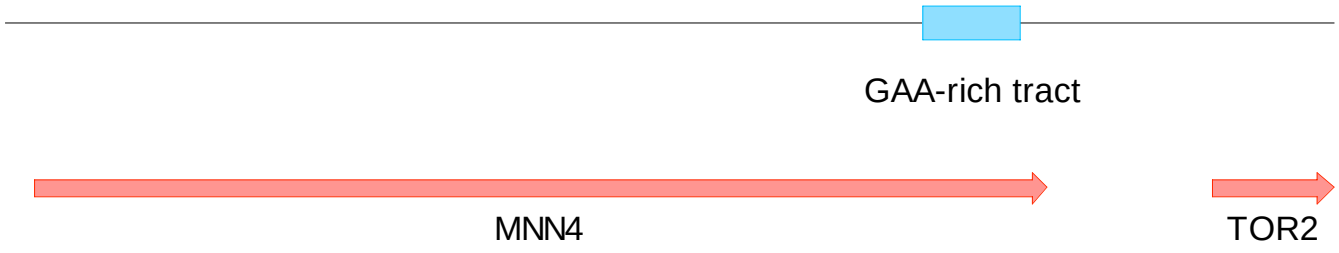


GAA/TTC repeats induce specific type of chromosomal rearrangements



GAA/TTC repeats induce specific type of chromosomal rearrangements





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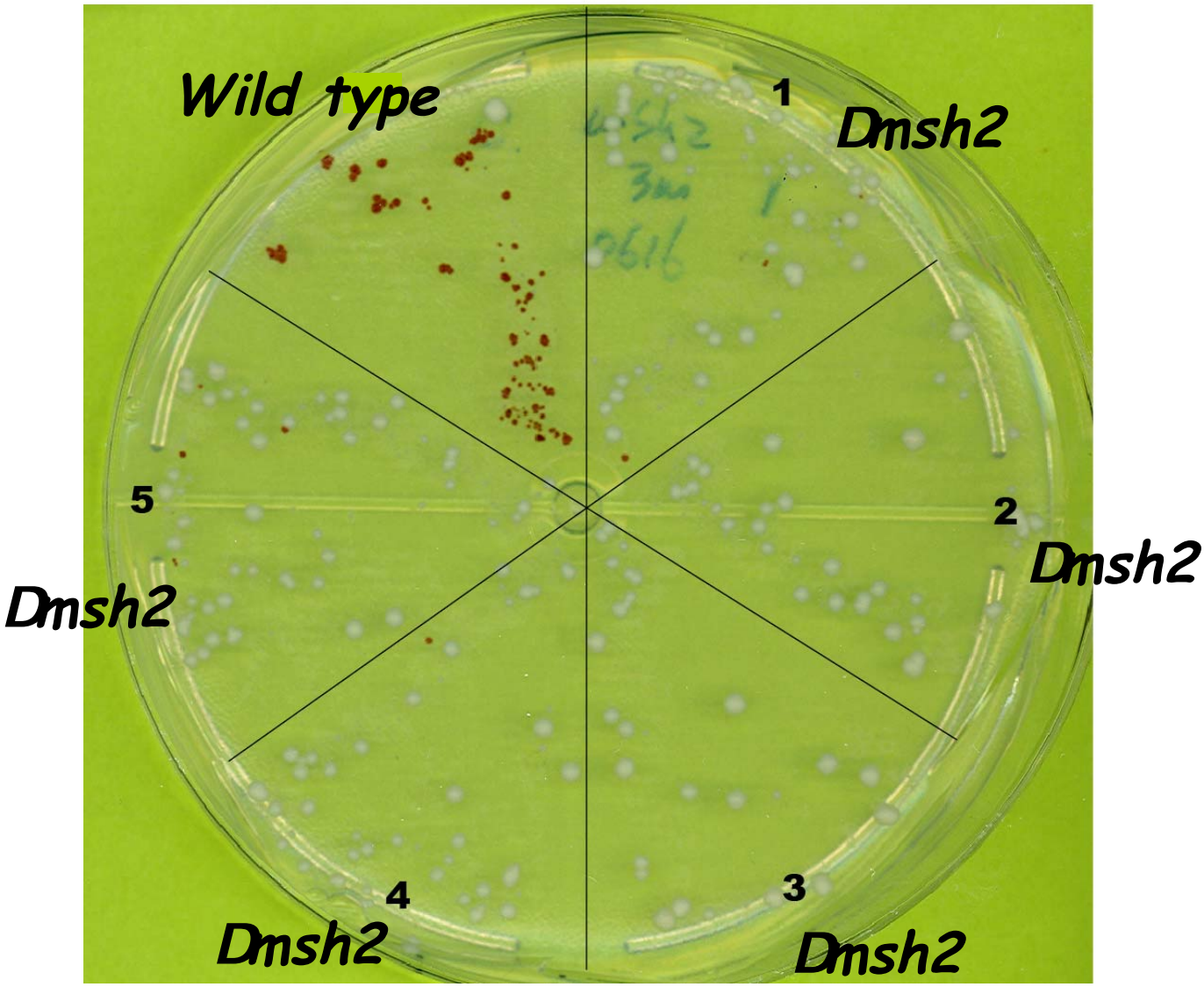
Induction of GCR events by GAA/TTC repeats depends on MMR machinery



Induction of GCR events by GAA/TTC repeats depends on MMR machinery

	Fold reduction (x)
wt	1x (7.4×10^{-5})
Dmsh2	16 x
Dpms1	15 x
Dmlh1	13 x

Induction of GCR events by GAA/TTC repeats depends on MMR machinery

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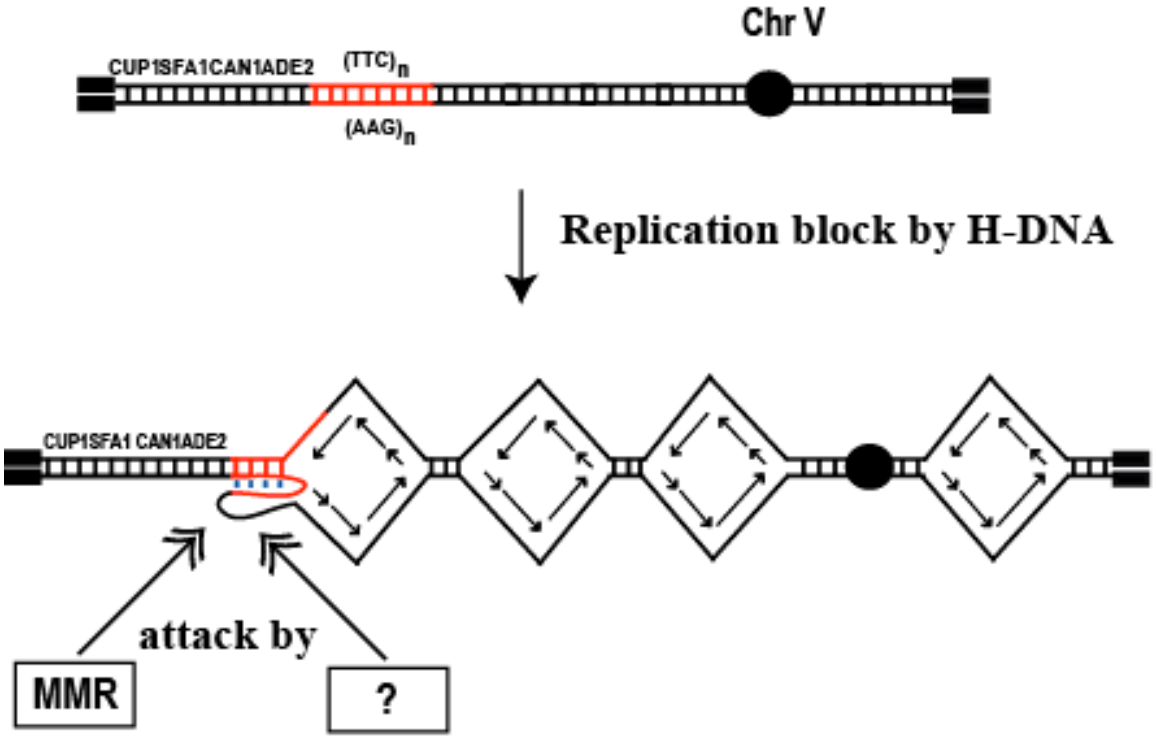
Dpms1	15 x
Dmlh1	13 x
pms1-G693A	13 x
msh2-G699N	13 x

Mechanism of MMR-dependent chromosomal fragility induced by GAA/TTC repeats



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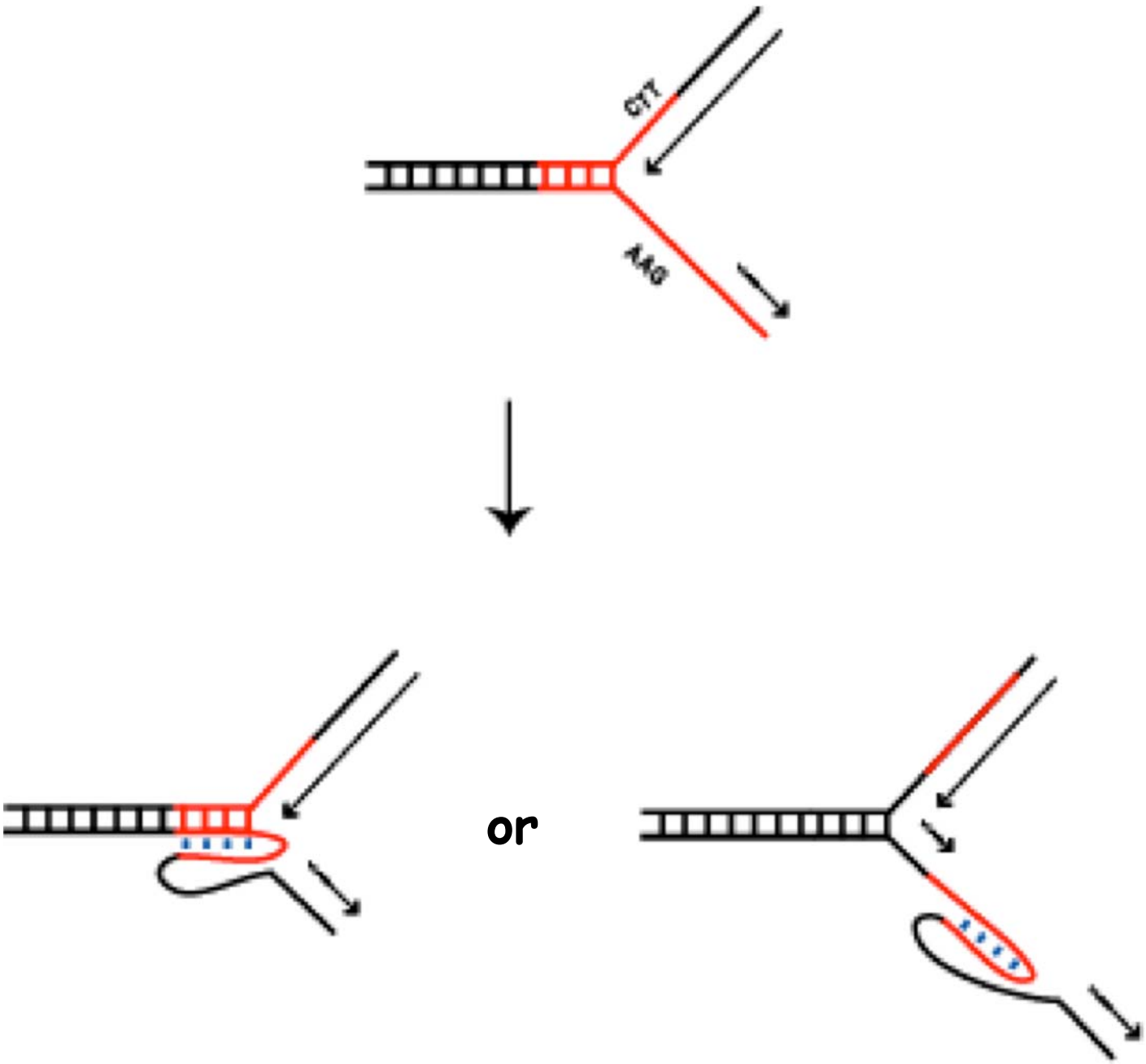

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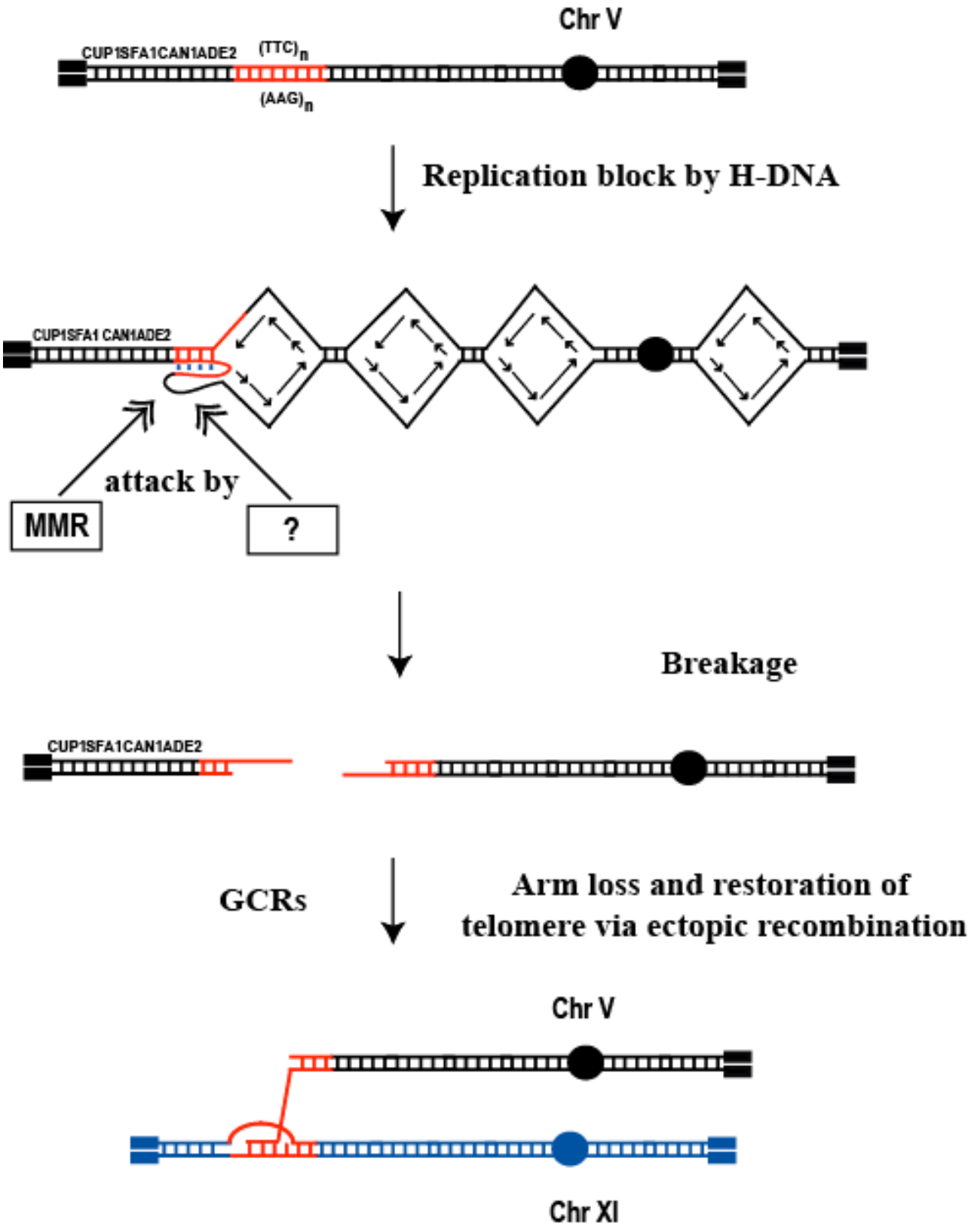
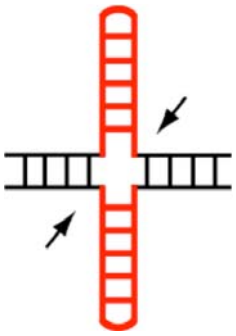


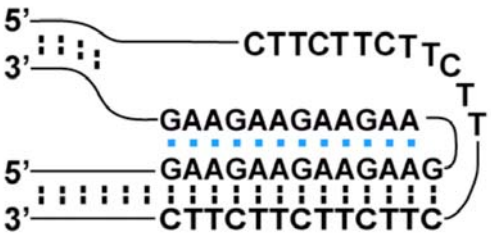
Central theme

Type of repetitive sequence determines type of the secondary structure

Inverted repeats



GAA triplet repeats



Pu Pu Py (Hu3)

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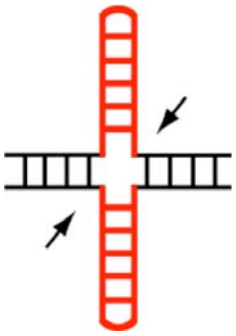
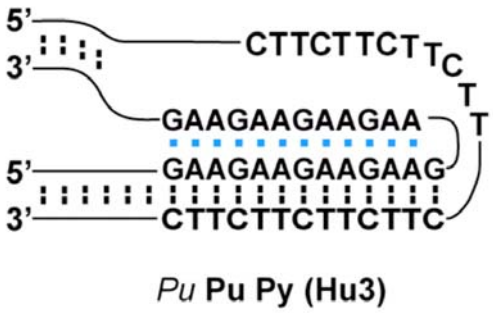


GAA triplet repeats



Secondary structure defines the mechanism of breakage, prone backgrounds and the structure of DSB ends

- cruciform resolution ???
- defects in DNA replication
- hairpin-capped DSBs

- substrate for MMR and ?
- defects in DNA replication
- GAA/TTC-terminated DSBs

Central theme

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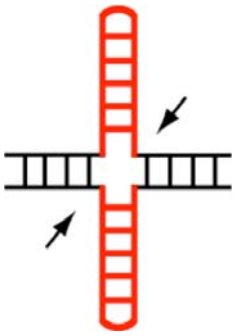

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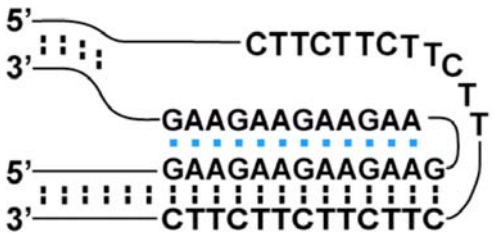

Pu Pu Py (Hu3)

Secondary structure defines the mechanism of breakage, prone backgrounds and the structure of DSB ends

- cruciform resolution ???
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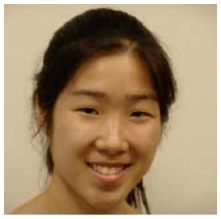
- substrate for MMR and ?
- defects in DNA replication
- GAA/TTC-terminated DSBs

Nature of DSB ends dictate the specific pattern of chromosomal rearrangements

- terminal deletions coupled with adjacent inverted duplications
- inverted DMs
- HSRs (inverted ladders)

- terminal deletions coupled with non-reciprocal translocations

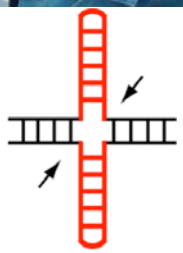
(non-homologous chromosome with expanded GAA-rich tracks is the donor)

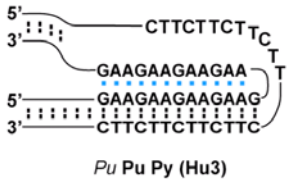


Clara Moon

George Lasker

Tamara Bodrogi





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Tufts University

Eric Alani, Jennifer Surtees
Cornell University

Anna Malkova
IUPUI

Rodney Rothstein
Columbia University

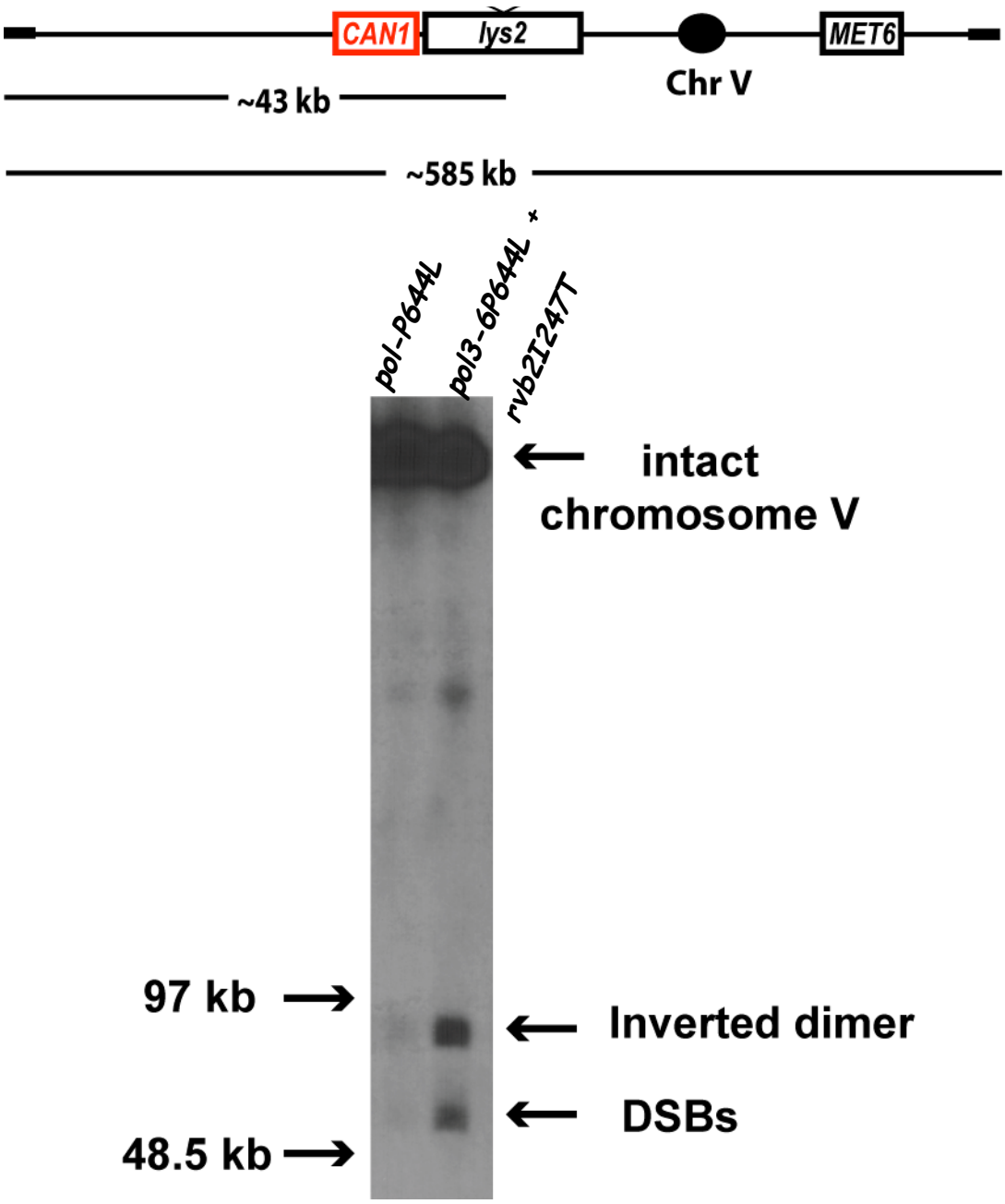
Anita Corbett, Milo Fasken
Katie Rudd
Emory Universi

Supported by: *NSF* *NIH/NIEHS*

Alula
DNA-LAND
Never odd or even
Too bad – I hid a boot
Cleveland DNA: Level C
A DNA Gun is in Uganda
Was it Eliot's toilet I saw?
Murder for a jar of red rum
May a moody baby doom a yam?
Go hang a salami; I'm a lasagna hog!
Satan, oscillate my metallic sonatas!
A Toyota! Race fast... safe car: a Toyota
Straw? No, too stupid a fad; I put soot on warts
Are we not drawn onward, we few, drawn onward to new era?
Doc Note: I dissent. A fast never prevents a fatness. I diet on cod
No, it never propagates if I set a gap or prevention
Anne, I vote more cars race Rome to Vienna
Sums are not set as a test on Erasmus
Kay, a red nude, peeped under a yak
Some men interpret nine memos
Campus Motto: Bottoms up, Mac
Go deliver a dare, vile dog!
Madam, in Eden I'm Adam
Oozy rat in a sanitary zoo
Ah, Satan sees Natasha
Lisa Bonet ate no basil
Do geese see God?
God saw I was dog
Dennis sinned
Don't nod

Gene Amplification Mediated By Palindromic Sequences

Mutations in RVB2 induce *Alu*-IR-mediated fragility



E.coli RuvABC

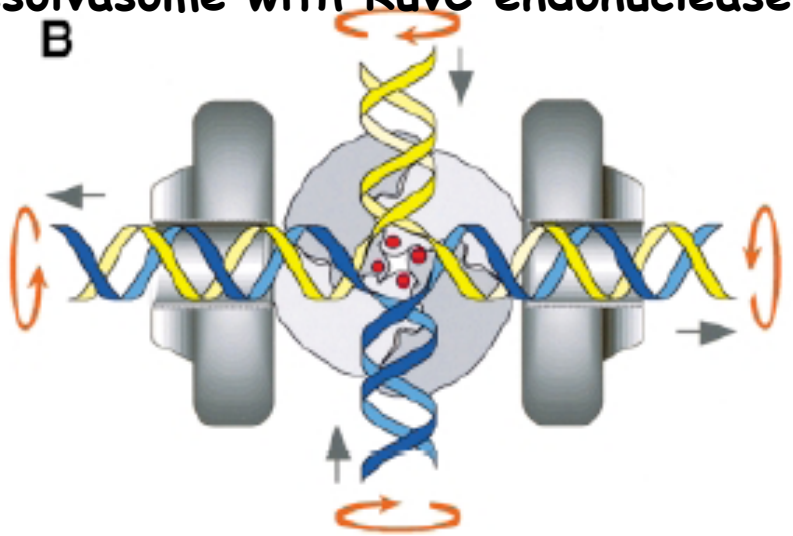
- Resolving Holliday junctions

- RuvA and RuvB are helicases

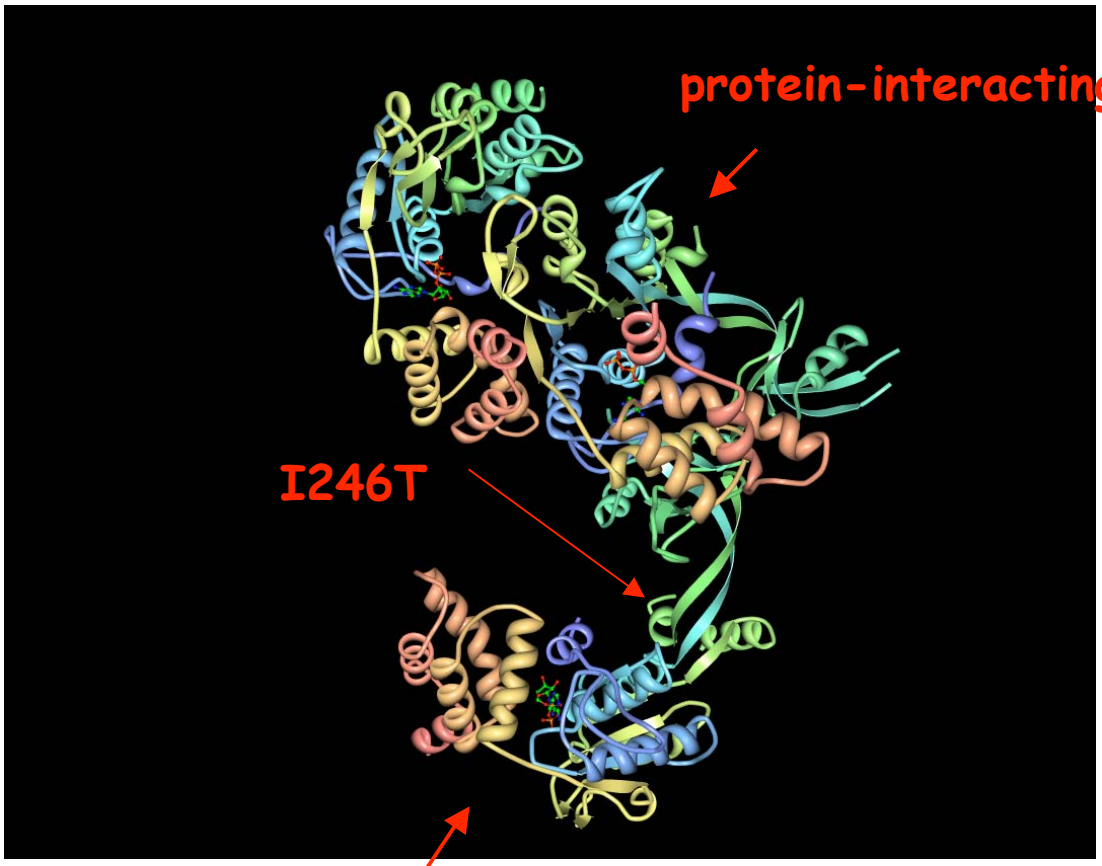
- RuvA tertamer recognizes and binds junctions

- Two RuvB hexameric rings bind diametrically opposite

- Form resolvasome with RuvC endonuclease



Crystal structure of human RuvBL1



Matias et al., JBC, 2006