

Damage-Induced Localized Hypermutability

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Damage-Induced Localized Hypermutability (LHM) in *Saccharomyces cerevisiae*

- Finding with small reporters (*Yang et al., PLoS Genetics 2008*)
- Large size of a region
- Genome-wide scale
 - *Multiple regions in a genome*
 - *Vast mutation clusters caused by chronic mutagenesis*

Mutations Can Alter Gene Function

Multiple Mutations are More Likely to Alter Gene Function

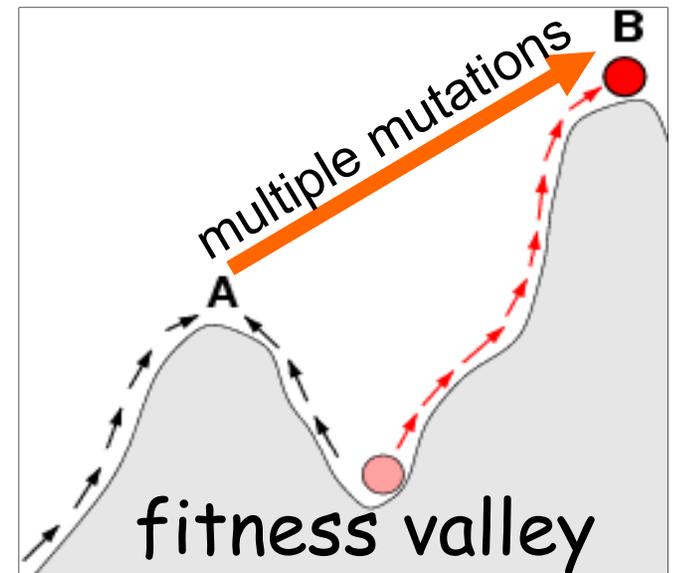
**Mutant
DNA**

Alleles with high fitness:
(enhanced or even new function)

- Evolution
- Ig-variants (SHM)
- Cancer (oncogenes)

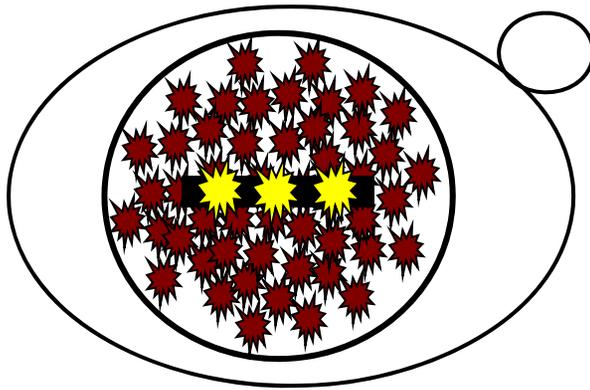
Inactivation or distortion of function:

- Genetic disease
- Cancer



Localized Hypermutability (LHM) Can Produce Alleles with Multiple Changes without Overloading the Rest of the Genome with Mutations

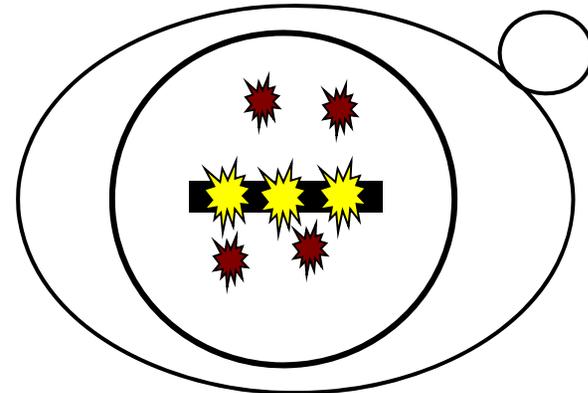
Genome-wide hypermutability



Catastrophic mutation load

LHM

Ig/SHM – programmed



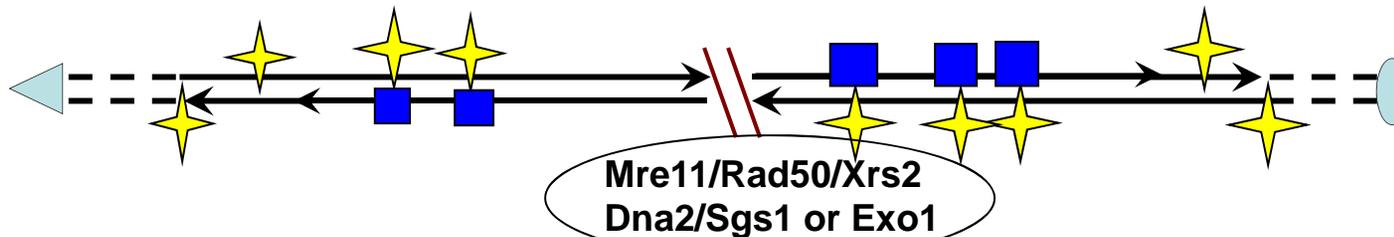
Mechanism for non-programmed?

Few additional mutations

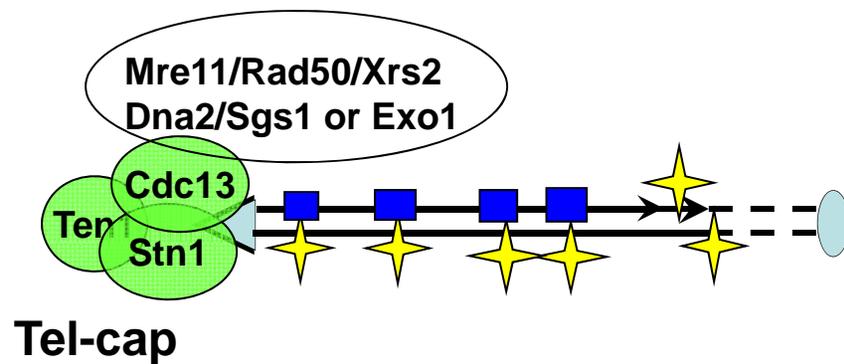
Hypothesis:

Error-prone Translesion Synthesis (TLS) in Damaged Long ssDNA can be a Source of Localized Hypermutability and Multiple Mutations

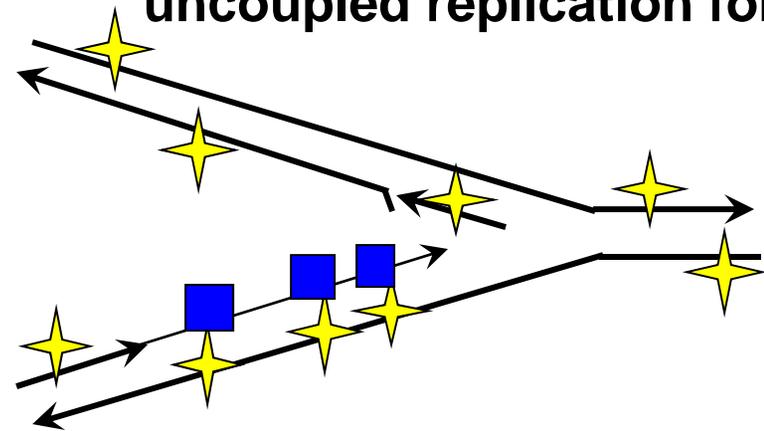
5'→3' resection at DSB



5'→3' resection at uncapped telomere

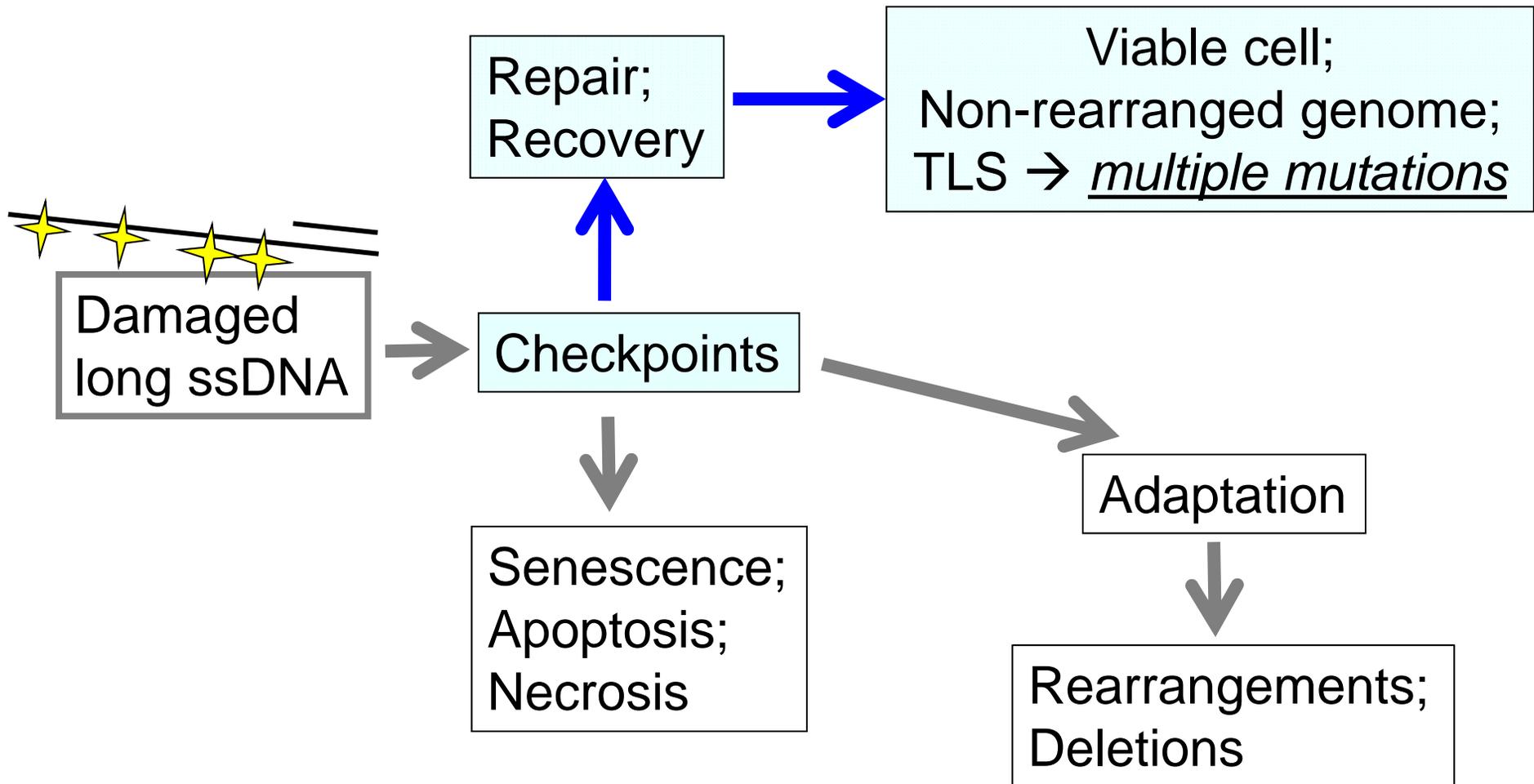


uncoupled replication fork



Checkpoints Are Triggered by Long ssDNA

How Efficient Would be Recovery and Repair of Damaged Long ssDNA?



Hypermutable of Damaged Single-Strand DNA Formed at Double-Strand Breaks and Uncapped Telomeres in Yeast *Saccharomyces cerevisiae*

Yong Yang, Joan Sterling, Francesca Storici[‡], Michael A. Resnick*, Dmitry A. Gordenin*

Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, North Carolina, United States of America

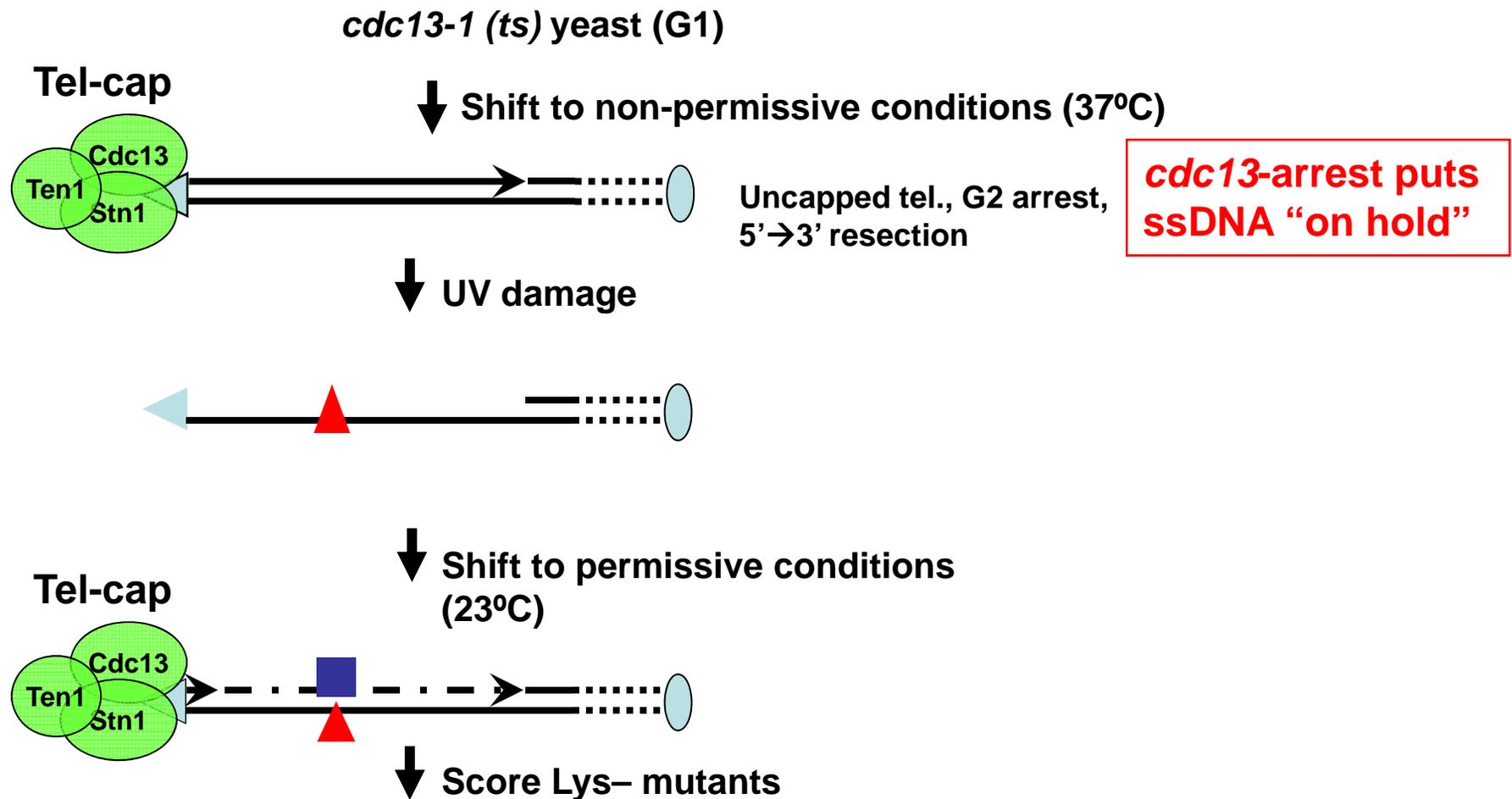
1. Create a region of persistent ssDNA *in vivo* (put ssDNA “on hold”)
2. Apply DNA damage
3. Restore to dsDNA at will
4. Count mutations in a reporter

Damage-Induced Localized Hypermutability (LHM) in *Saccharomyces cerevisiae*

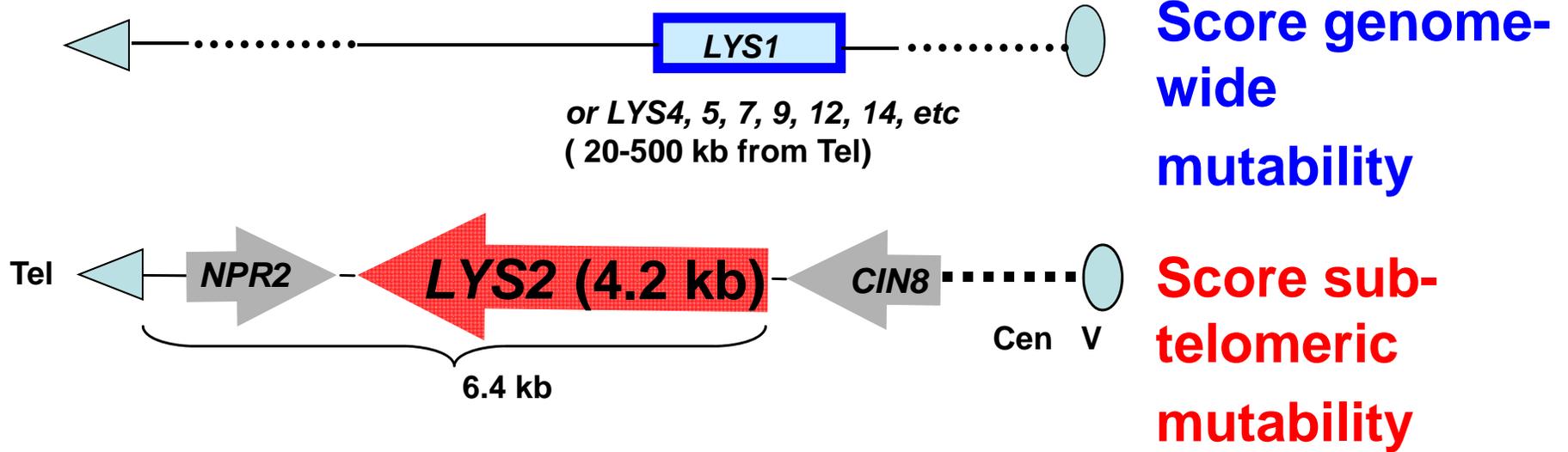
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1. Create a region of persistent ssDNA
2. Apply DNA damage
3. Restore to dsDNA
4. Count mutations in a reporter

Yeast telomere



Sub-Telomeric *LYS2* Reporter

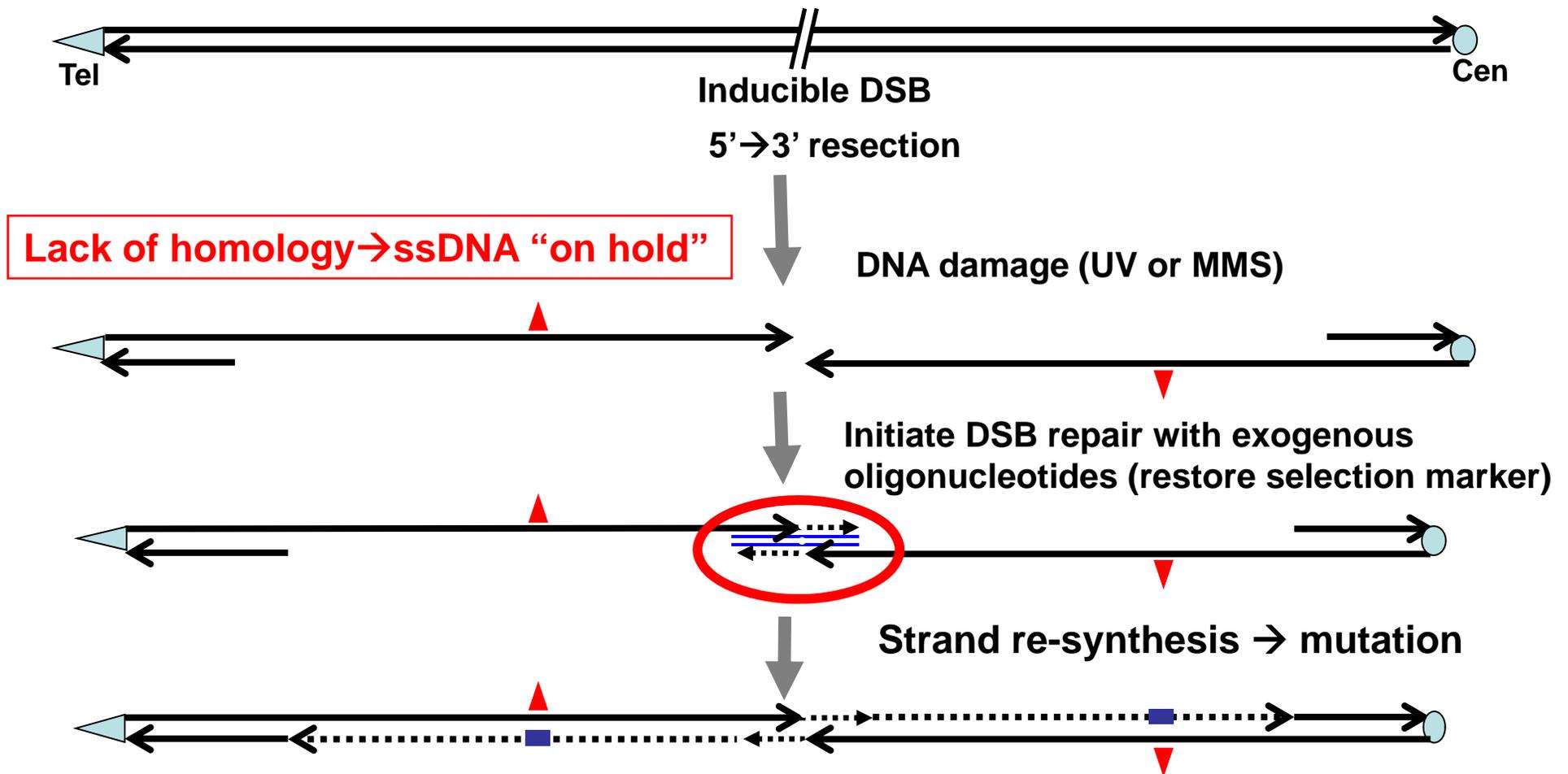


UV-mutagenesis ($\times 10^4$)

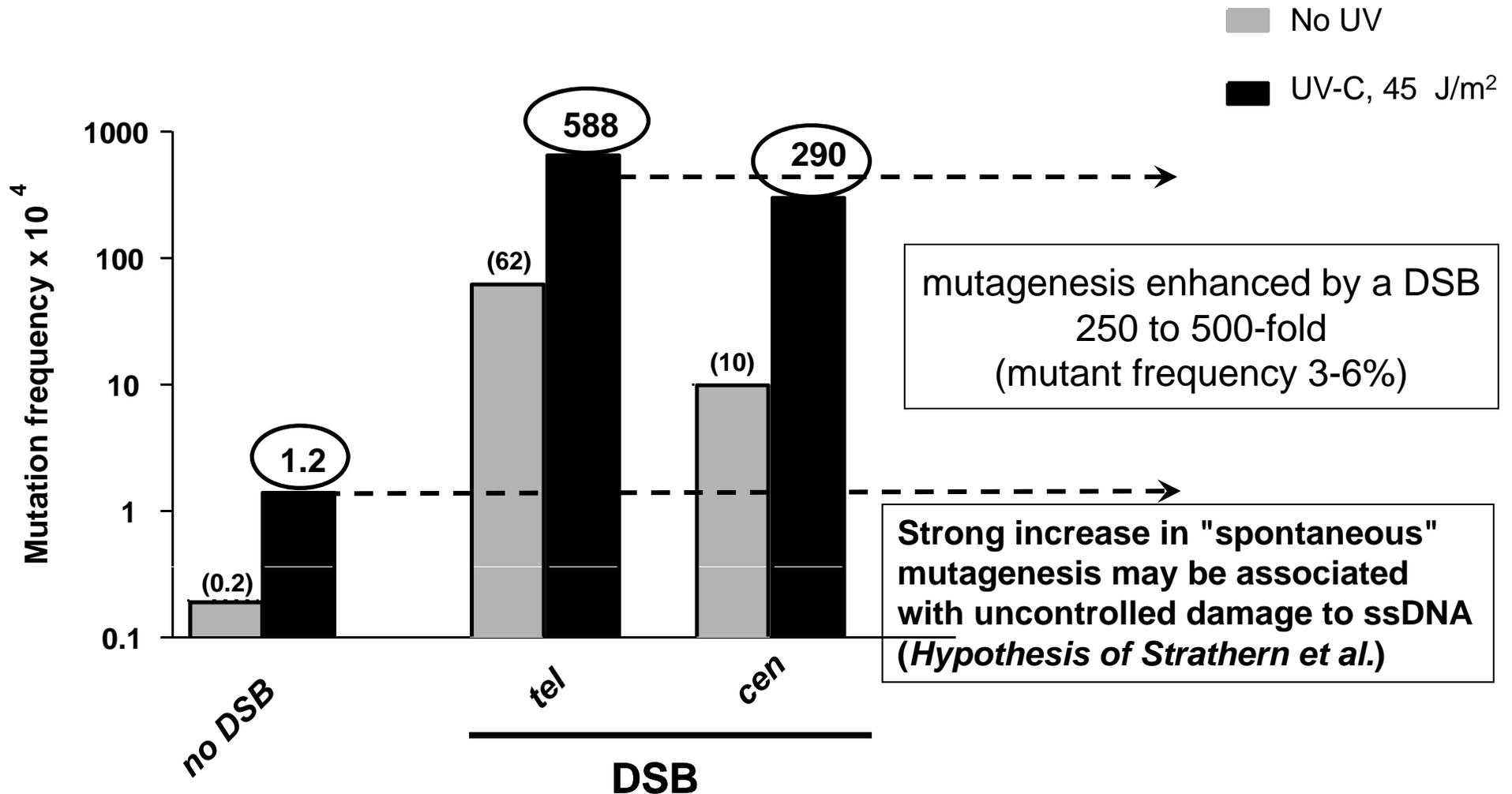
Tel. ssDNA (<i>cdc13</i> -arrest)	Genome-wide (<i>Lys</i> ⁻ , <i>LYS2</i> ⁺)	Sub-telomeric (<i>lys2</i> ⁻)
-	4	3
+	5	182

UV – 45 J/m²;
survival ~50%

Mutagenesis in Transient ssDNA Around a DSB



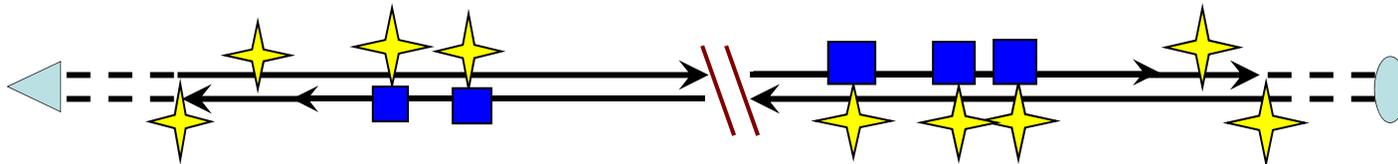
UV-Induced Hypermutagenesis in the Vicinity of a DSB



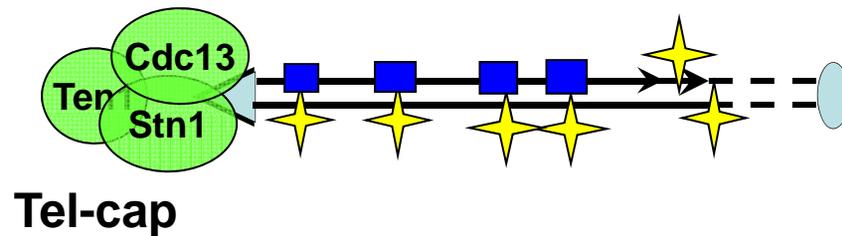
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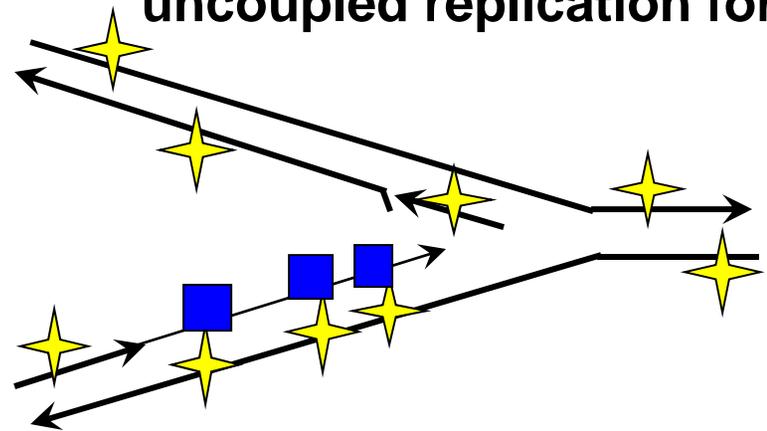
5'→3' resection at DSB



5'→3' resection at uncapped telomere

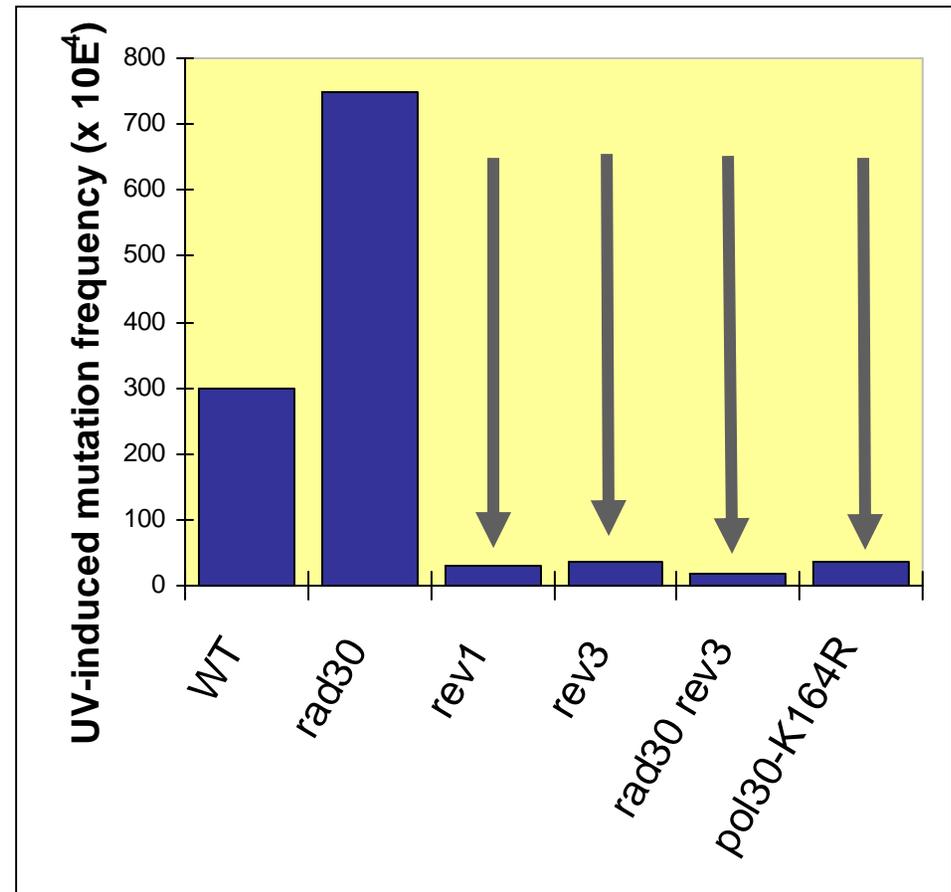
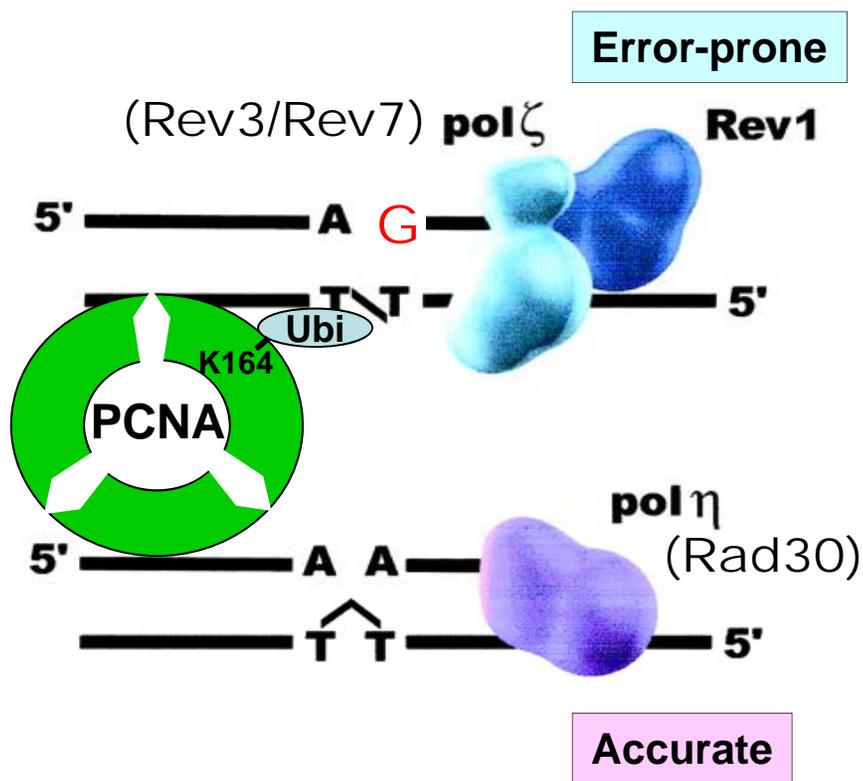


uncoupled replication fork



Error-Prone Translesion Synthesis (TLS) Relies on Pol ζ /Rev1 and PCNA-monoubiquitination at K164

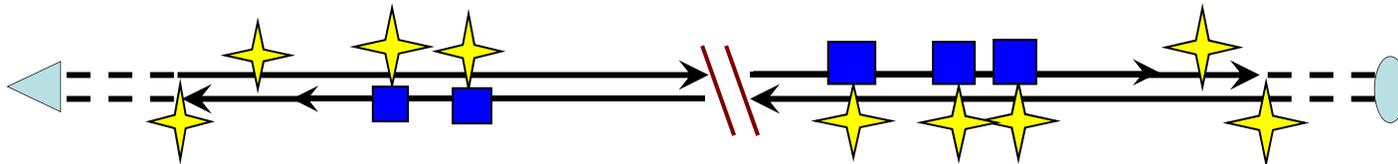
Hypermutable Requires Same Factors as Error-Prone TLS (TLS)



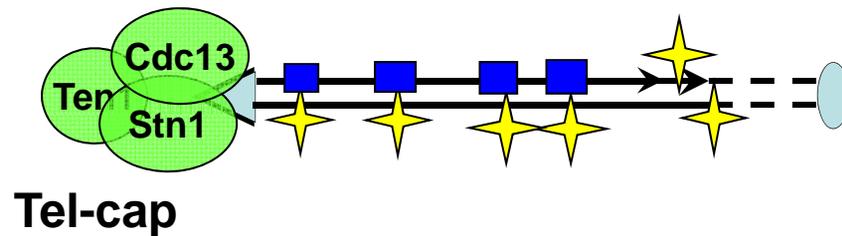
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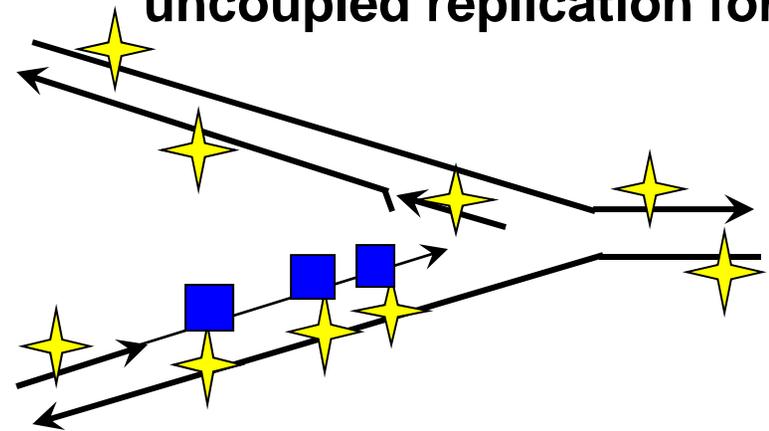
5'→3' resection at DSB



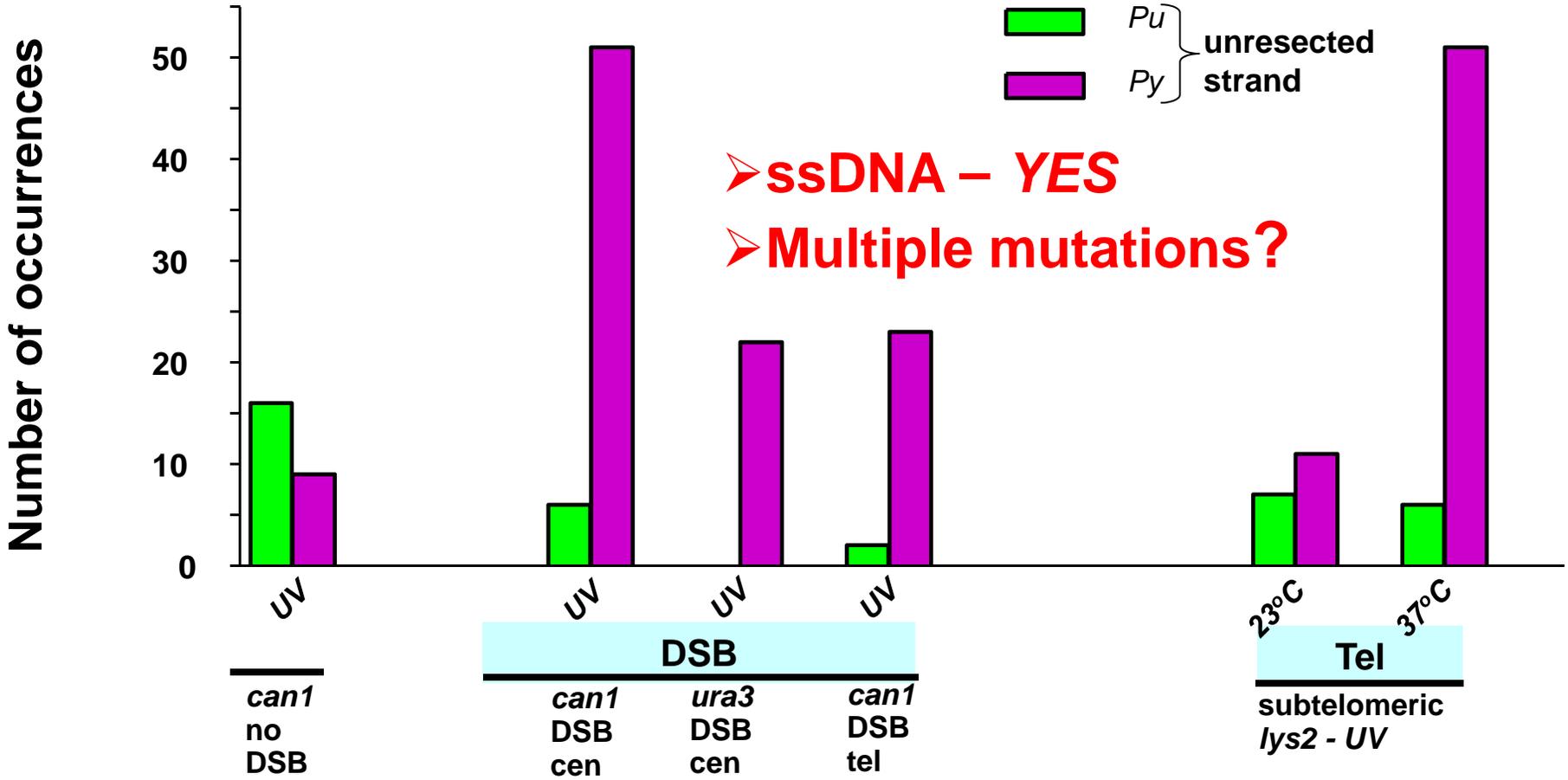
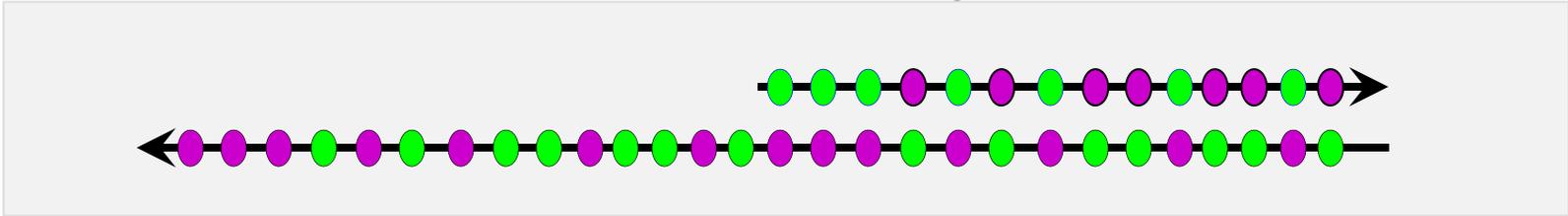
5'→3' resection at uncapped telomere



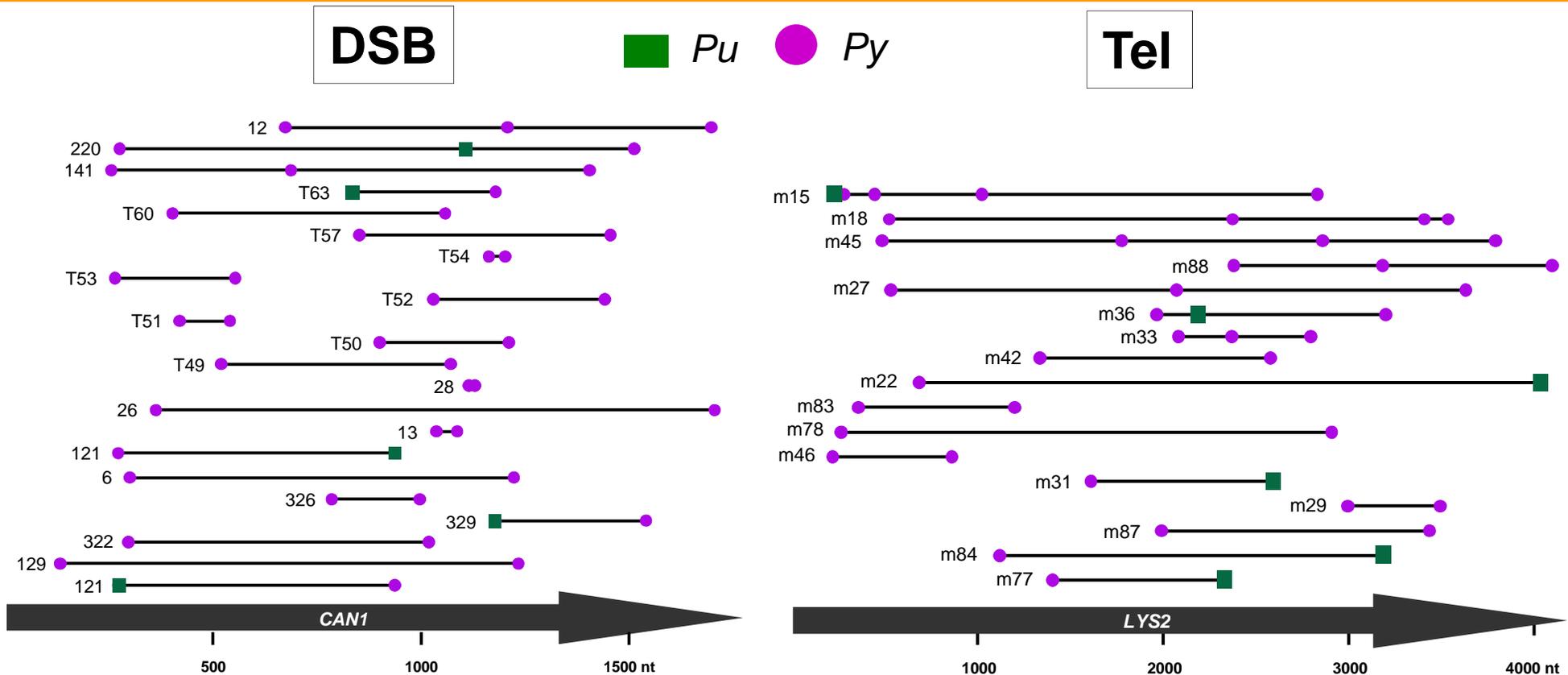
uncoupled replication fork



Strong Strand Bias to Pyrimidines in the Non-resected Strand Indicates that Mutations Result from UV-photoproducts in ssDNA.



UV-induced mutants generated via ss-DNA (DSB and Tel) carry widely separated strand-biased multiple mutations



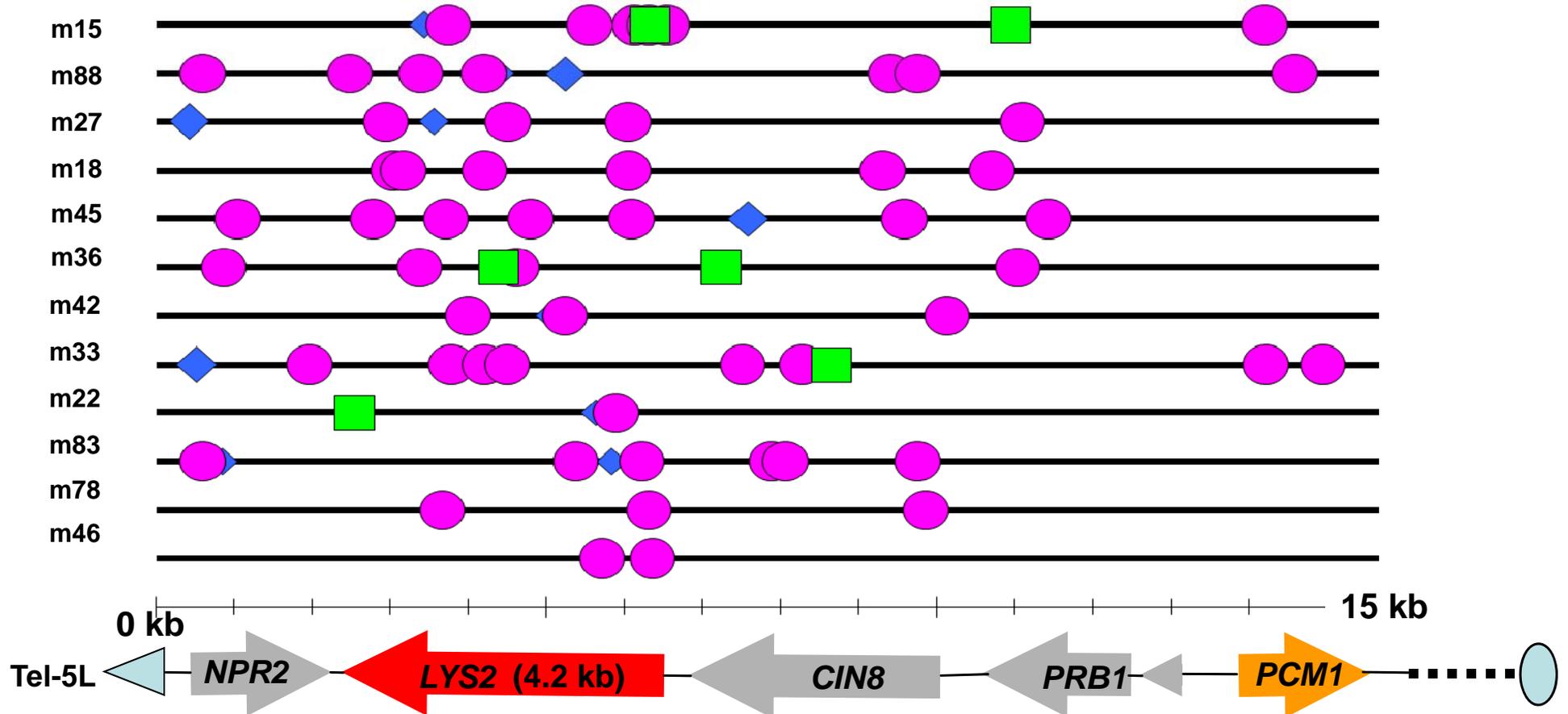
~ 0.5 x 10⁻³ per nt (1,000-fold compared to genome-wide)

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- Finding with small reporters (*Yang et al., PLoS Genetics 2008*)
- Large size of a region
- Genome-wide scale
 - Multiple regions in a genome
 - Vast mutation clusters caused by chronic mutagenesis

G2-arrested cells (long subtelomeric ssDNA) – up to 11 mutations spanning over 15 kb
 G1-cells (no subtelomeric ssDNA) – only one mutation in each of 9 mutants

Mutation bias to pyrimidines marks the areas of damaged ssDNA



indel; complex	Py	Pu
18% (14)	74% (57)	8% (6)

Large Area with Multiple Mutations Associated with Damaged ssDNA

Areas of multiple mutations could cover:

- ORFs, large exons or large domains ~ 1 kb+
- Adjacent exons ~ 10 kb+
- Small genes (e.g. *TP53*, *EGFR-201*, *CDK2*, *p21*, *RAD6*) ~ 20 kb+
- Large genes ~ 100 kb+

Damage-Induced Localized Hypermutability (LHM) in *Saccharomyces cerevisiae*

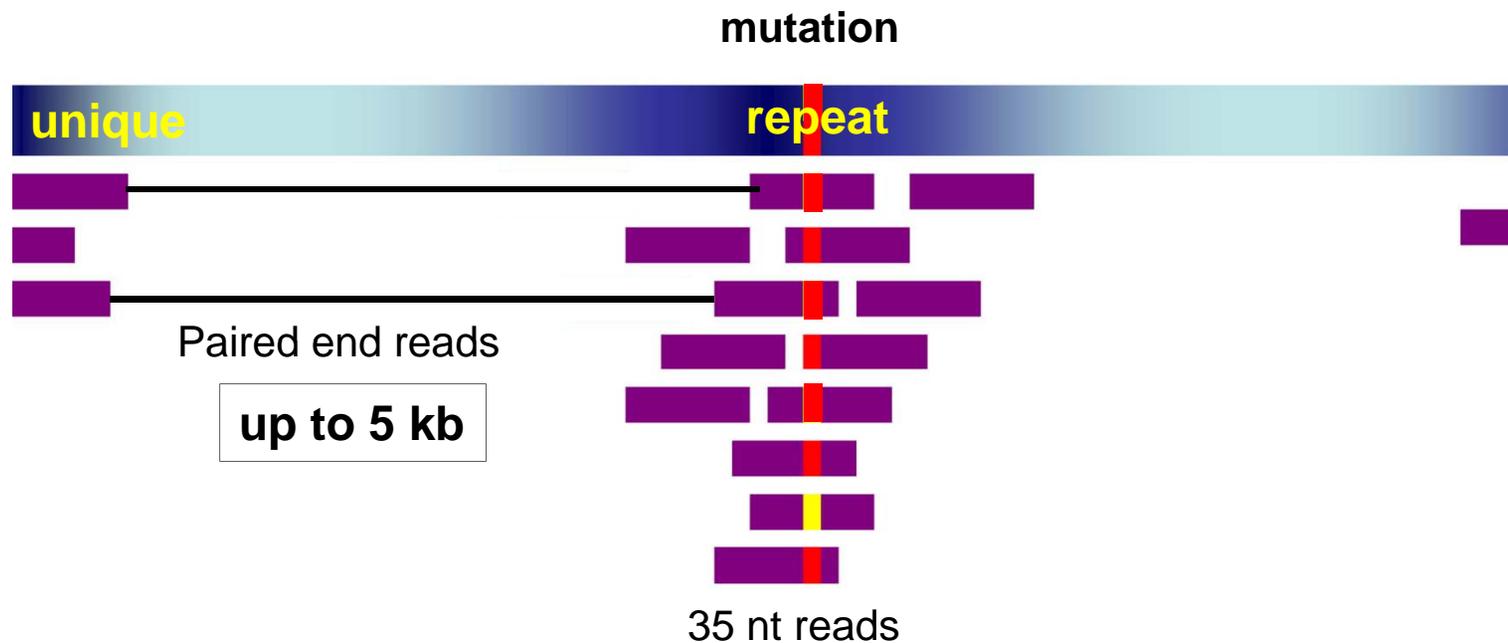
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Genome-wide search.

Collaboration with Piotr Mieczkowski,

-- UNC High-Throughput Sequencing Center

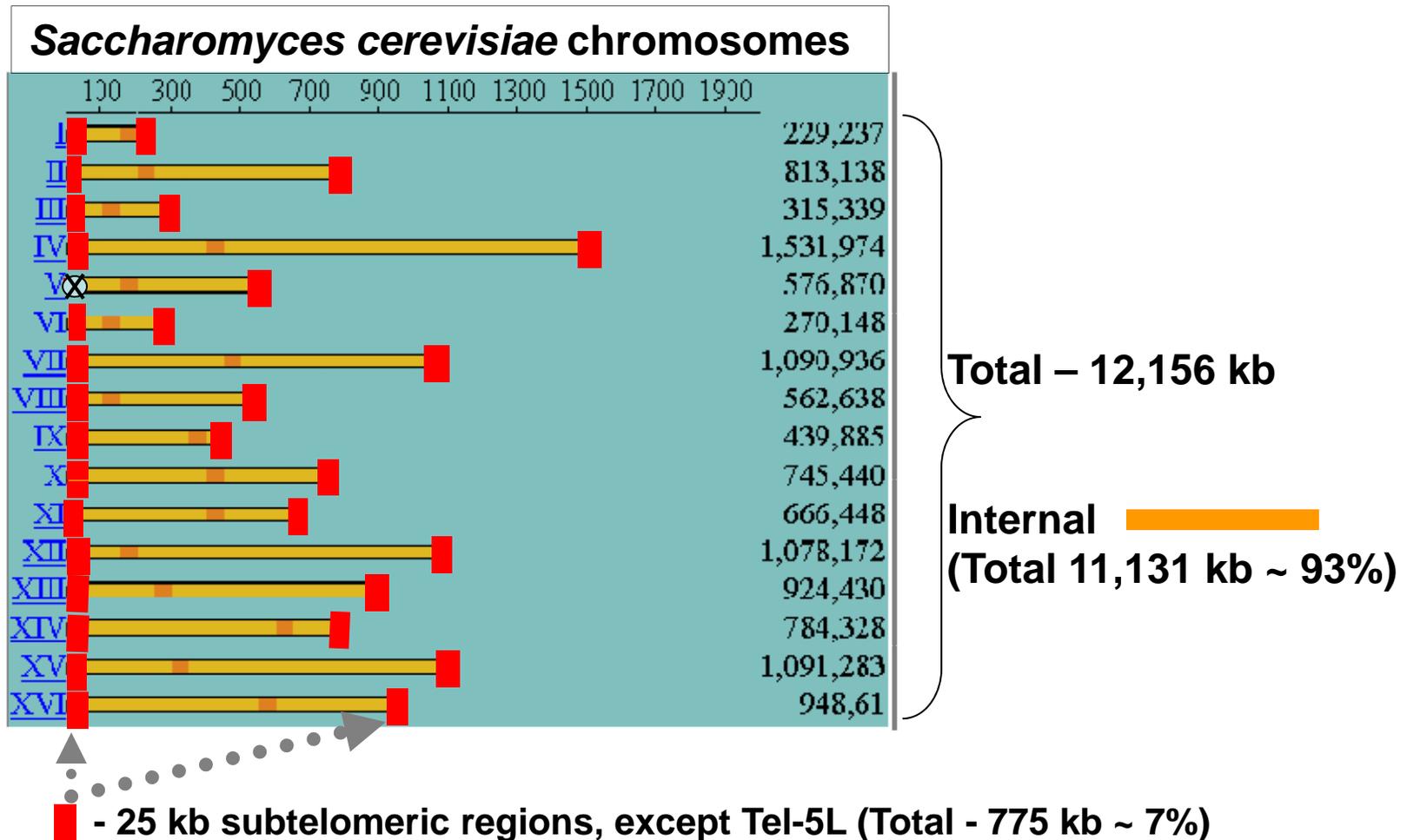
Identifying and Mapping Polymorphisms



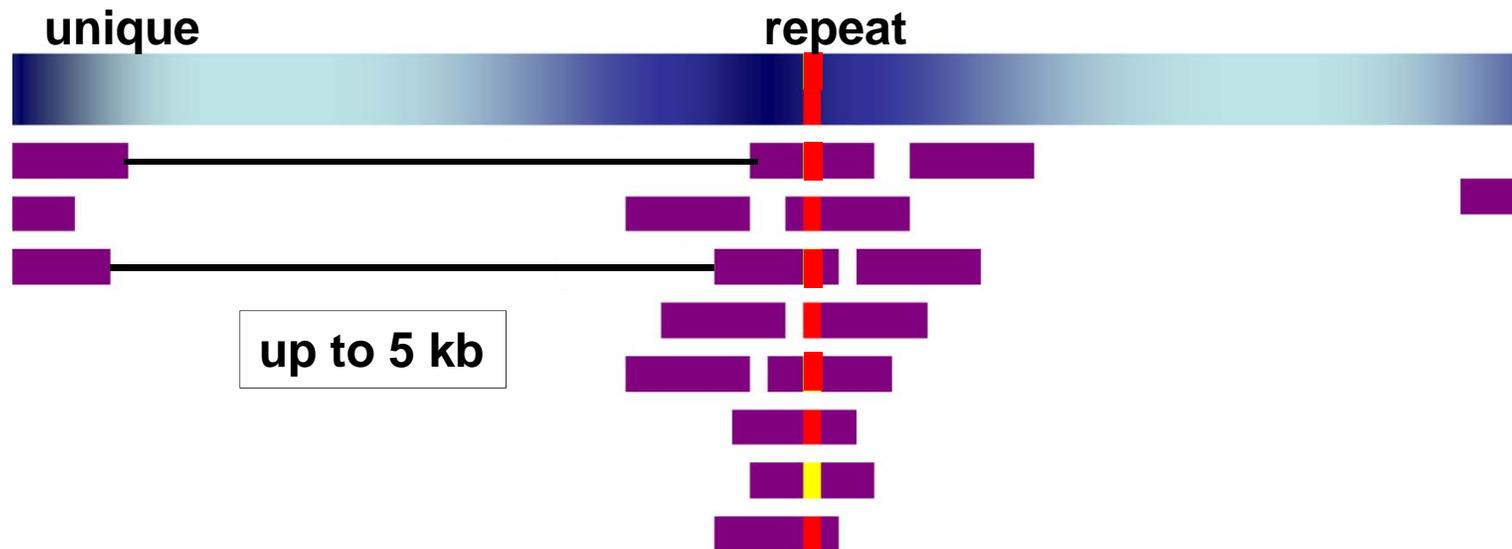
-- Illumina sequencing can detect as little as 10 damage-induced mutations in a yeast genome

-- *CLC Bio* – a biologist-friendly software

Count Base Substitutions in Subtelomeric and Internal Regions of Chromosomes



Identifying and Mapping Mutations



Paired End Technology Can Solve the Ambiguity for a Part of Repeats

Will it find mutations in subtelomeric regions containing homology blocks?

Predictions for UV-induced mutations in G2-arrested *cdc13-1* cells:

- Subtelomeric clusters
- Increased probability of subtelomeric mutations
- Strand bias with subtelomeric mutations

First Indication: Subtelomeric Clusters – Higher Incidence in G2-arrested *cdc13-1* Cells

G2-arrested *cdc13-1* cells (37°C)

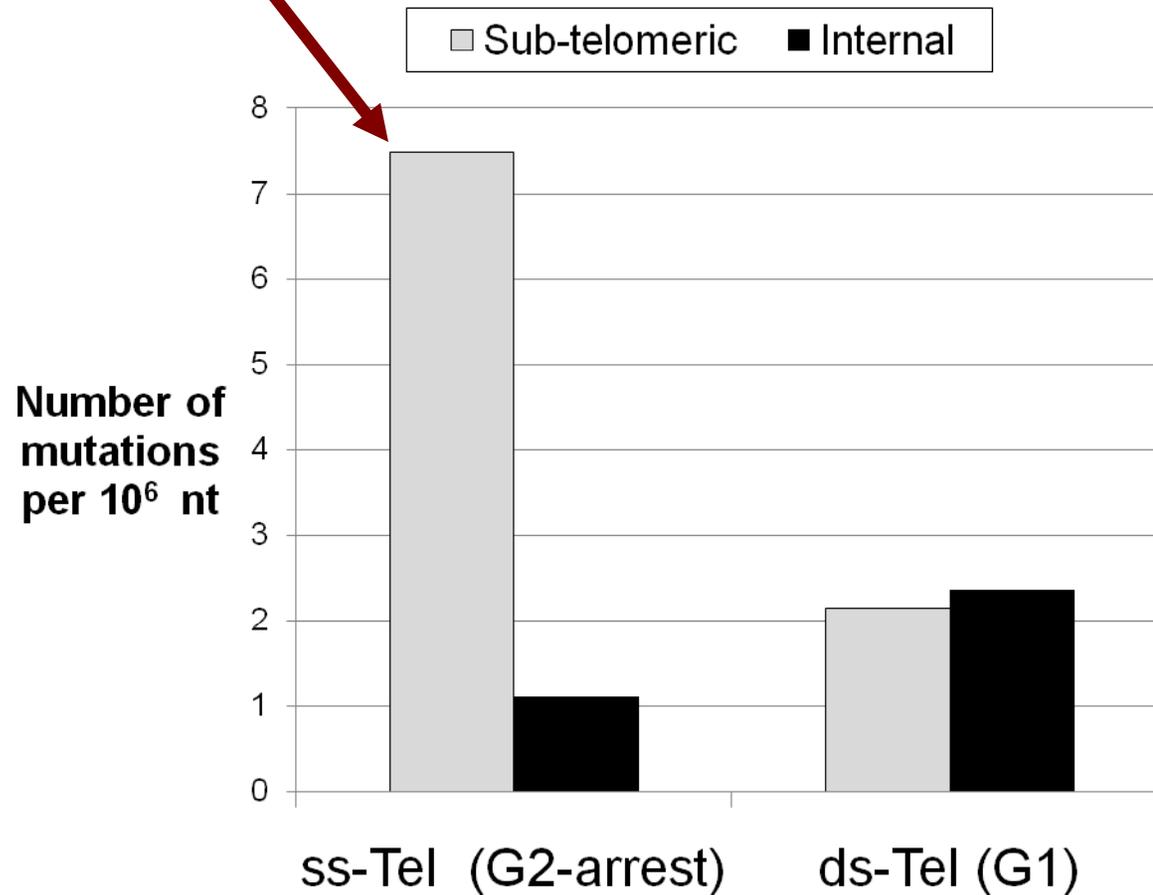


Genome of a mutant	I		II		III		IV		V		VI		VII		VIII		IX		X		XI		XII		XIII		XIV		XV		XVI	
	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R		
m15					1				8			1	1								2			1						1		
m33									9					4																		
m45	1		1				1		7																							
m83				1					8	2		1	1				1									1						
m88									7											3			1				4					

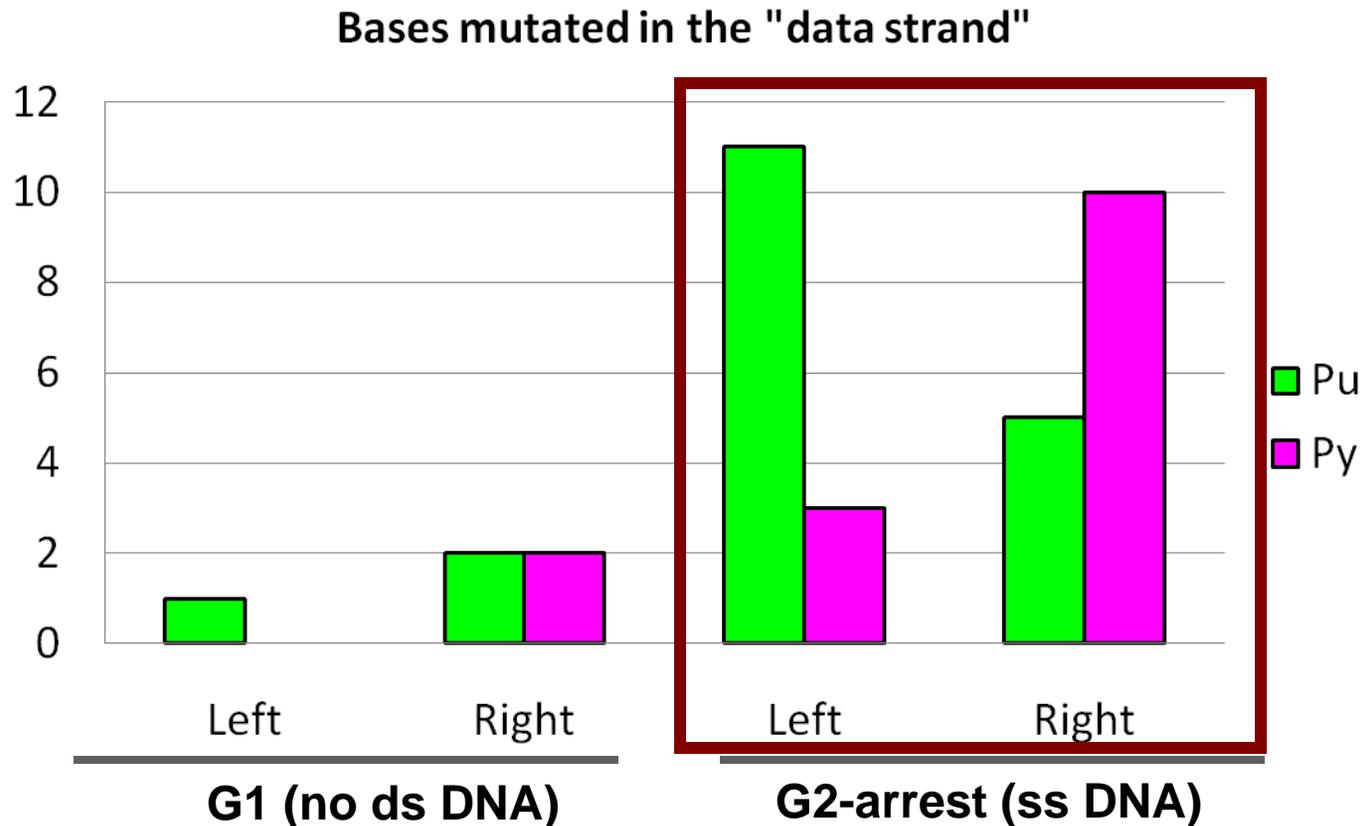
G1 *cdc13-1* cells (23°C)

Genome of a mutant	I		II		III		IV		V		VI		VII		VIII		IX		X		XI		XII		XIII		XIV		XV		XVI	
	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R		
m2									1		1																					
m4									1								1													1		
m7									1										1							1						

Increased Probability of UV-Induced Subtelomeric Mutations in G2-arrested *cdc13-1* Cells



Strand Bias as Expected



High Frequency of UV-induced Strand-Biased Multiple Mutations Associated with Transient ssDNA

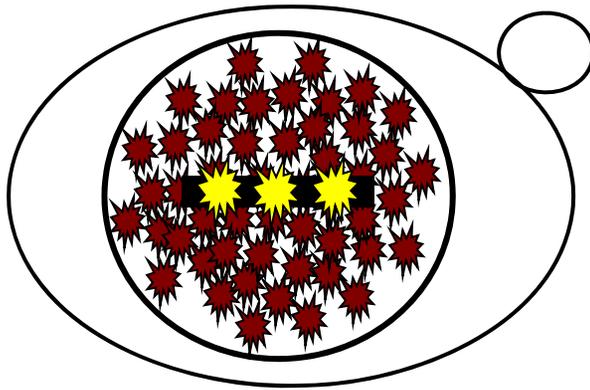
- **Density of UV-induced mutation ~ 0.5×10^{-3} per nt (1,000-fold compared to genome-wide)**
- **Damage-induced hypermutability (UV and MMS) of ssDNA is under Pol ζ control**
(Rev3, Rev1, PCNA-K164-Ubi)
- **Single area of hypermutability spans up to 15 kb**
- **Cell can tolerate several simultaneous areas of UV-induced LHM**

- Genome-Wide Hypermutable – *intolerable mutation load*
- Localized Hypermutable (LHM) – *escape high mutation load*

➤ in artificial long ssDNA – YES

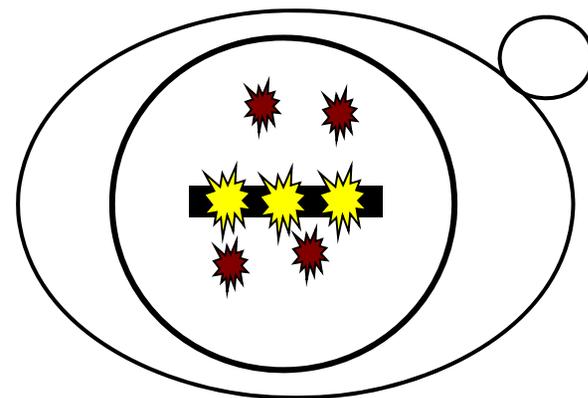
➤ in "natural" conditions???

Genome-wide
mutagenesis
"by a textbook"



Catastrophic
mutation load --
~ 1,000 additional
mutations

LHM



Viable cells
 ≤ 10 additional
mutations

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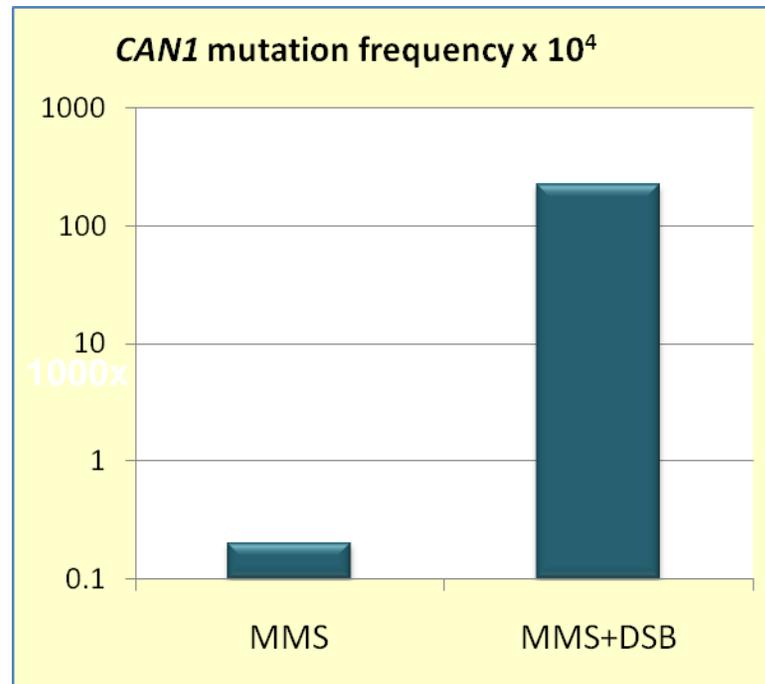
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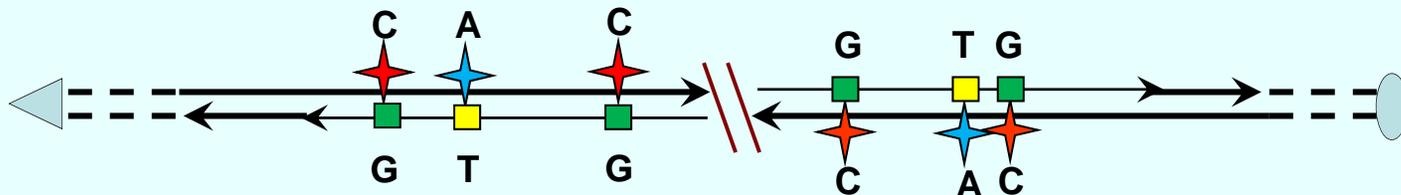
-- UNC High-Throughput Sequencing Center

MMS-induced hypermutability in ssDNA around an I-SceI-DSB (*Yang et al.*):

- mostly substitutions in cytosines (also some substitutions in adenines);
- in agreement with 3meC and 1meA (or 3meA) being primary mutagenic lesions



MMS induced mutations in the area of 5'→3' resection at DSB

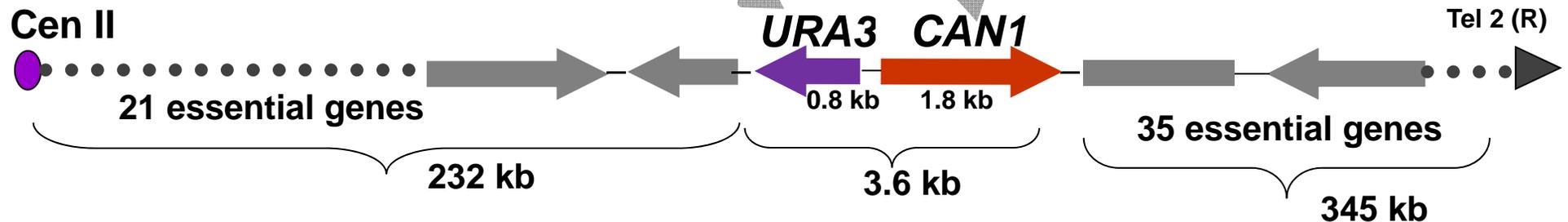


Detecting Transient Regions of MMS-Induced LHM

- closely spaced *CAN1* and *URA3* in chromosome II – to screen for closely spaced multiple mutations
- surrounded by essential genes – to eliminate GCR

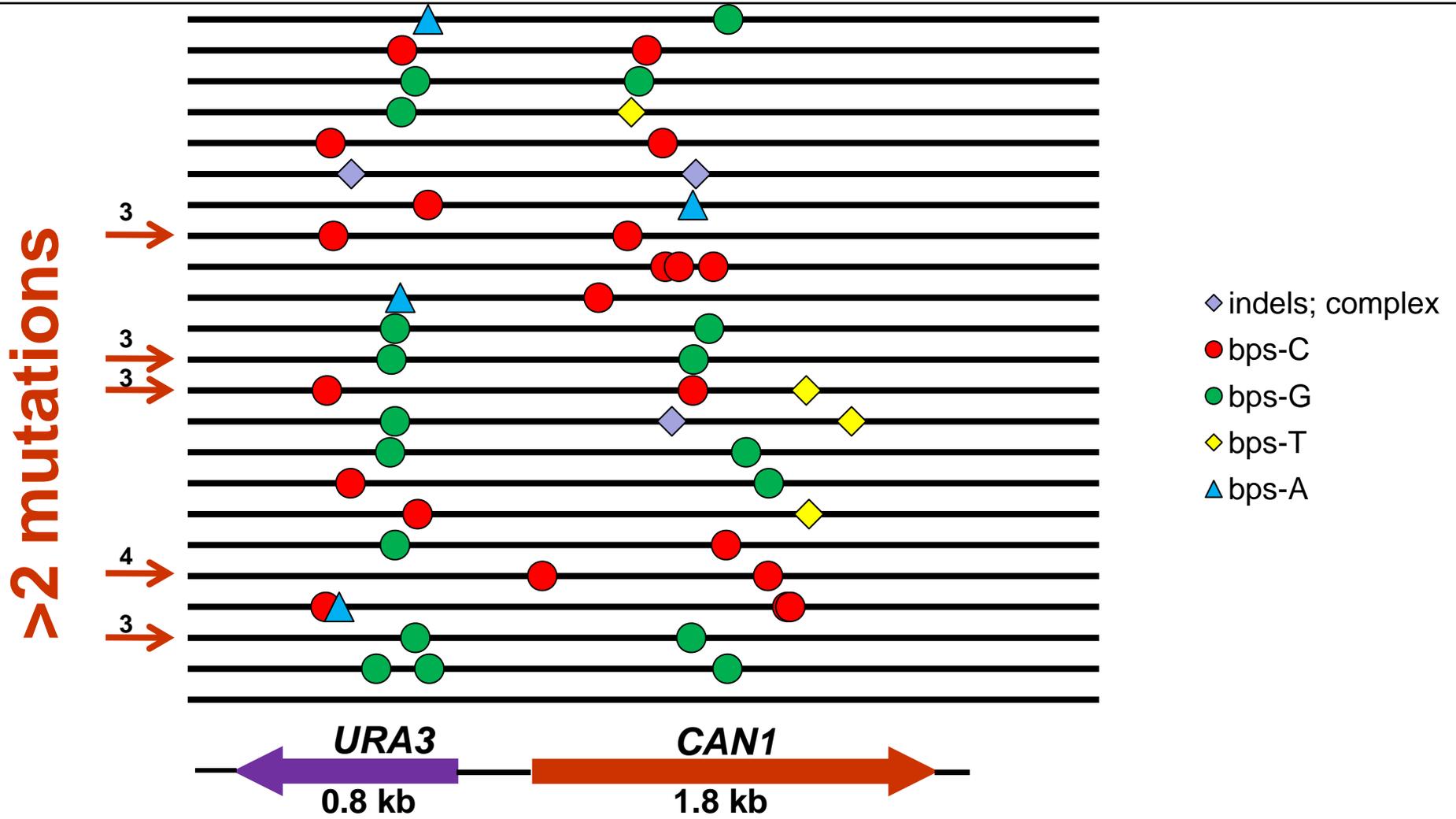
Hypothesis:

Double inactivation of *URA3* (5FOA-R) and *CAN1* (Can-R) would be often caused by transient localized hypermutability



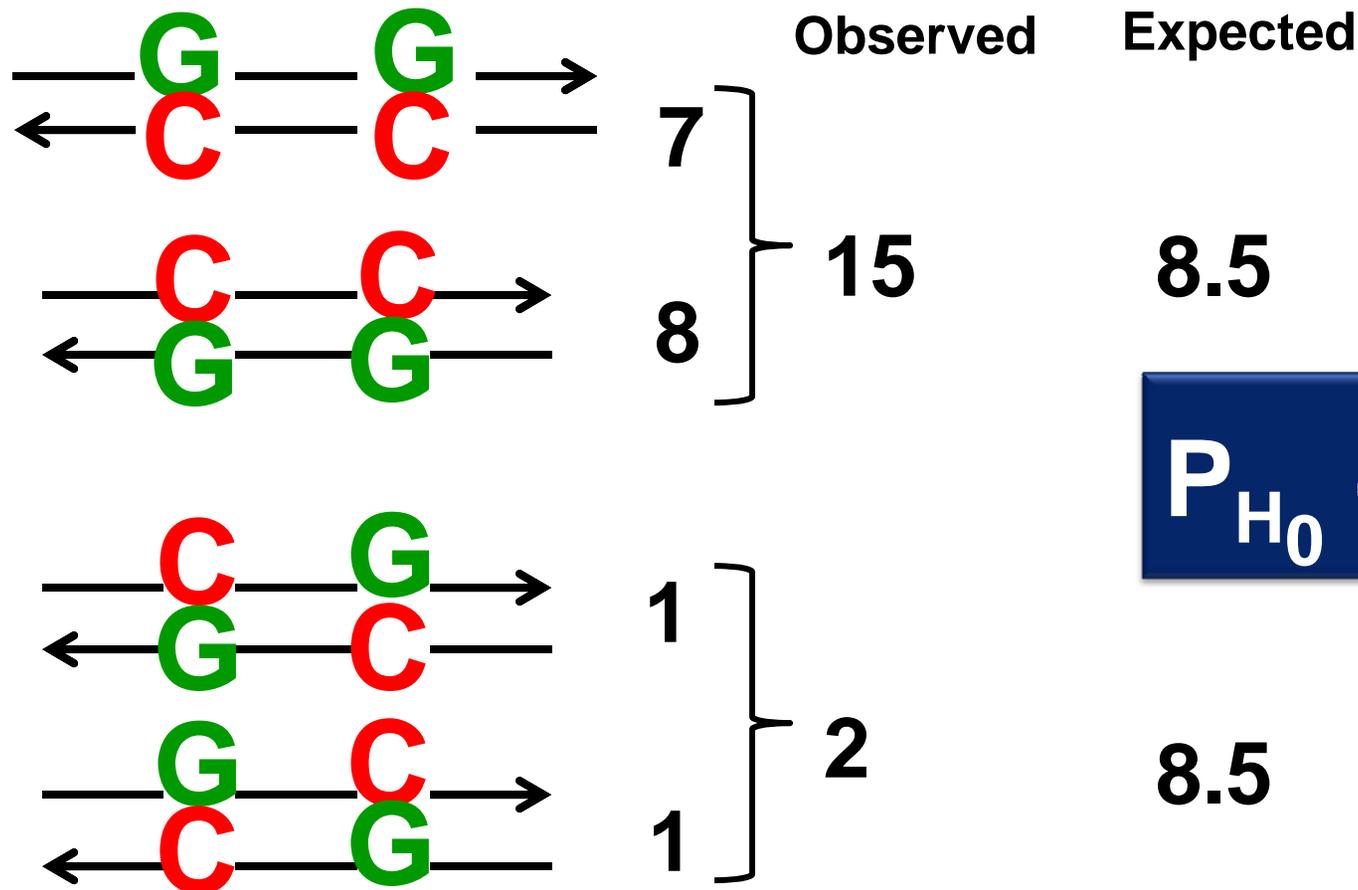
MMS-Induced *can1 ura3* Often Carry More than Two Mutations

16 out of 22 *can1 ura3* mutants fit specificity determined for ssDNA --
-- [C, C+A] or [G, G+T] mutated in the same strand -- **strand-coordinated clusters**



MMS-Induced Double and Multiple Mutants in Closely Spaced *CAN1 URA3* are Enriched with Strand-Coordinated Mutation Pairs

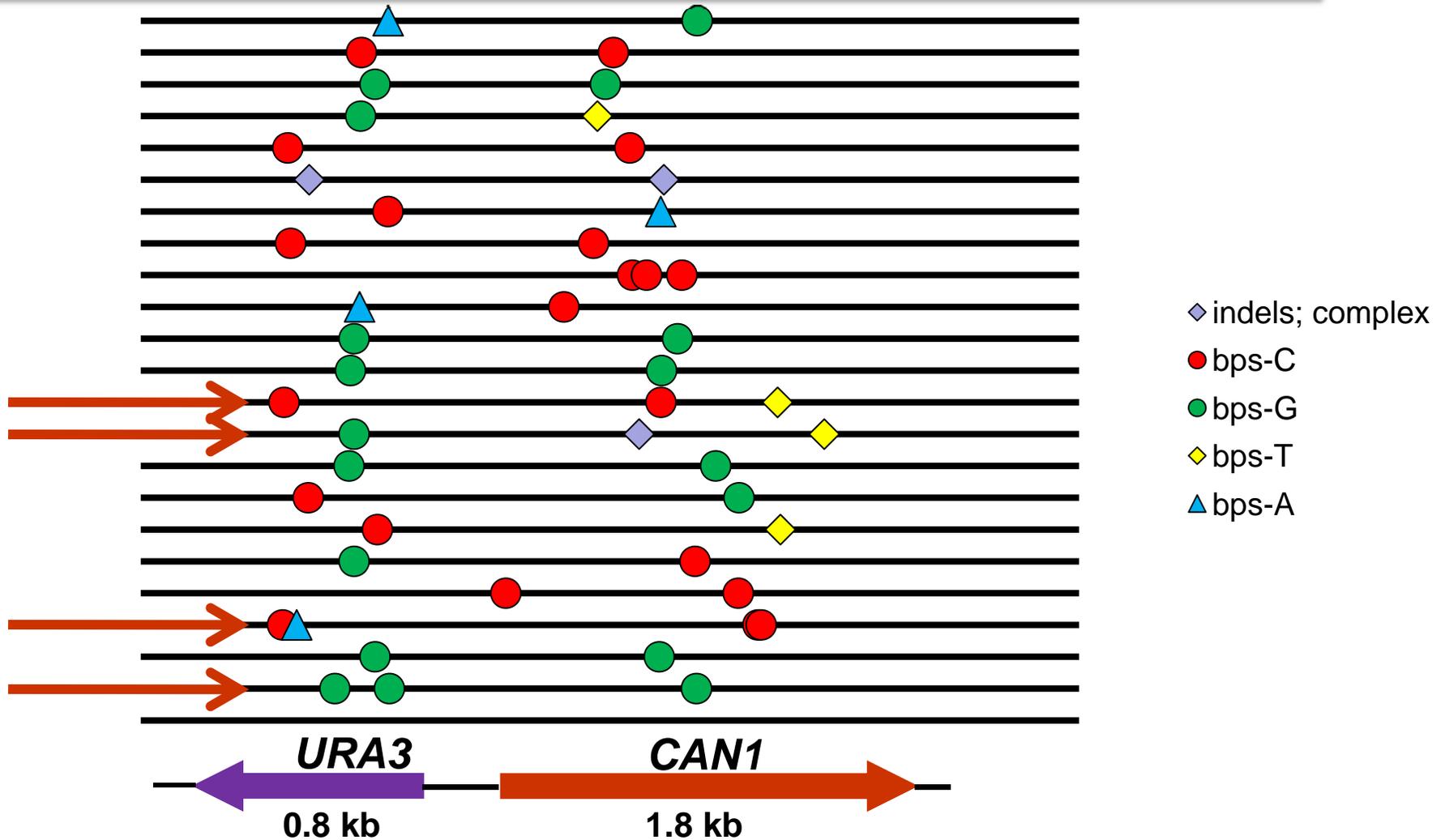
G-G and C-C are the most frequent neighbors at the same strand



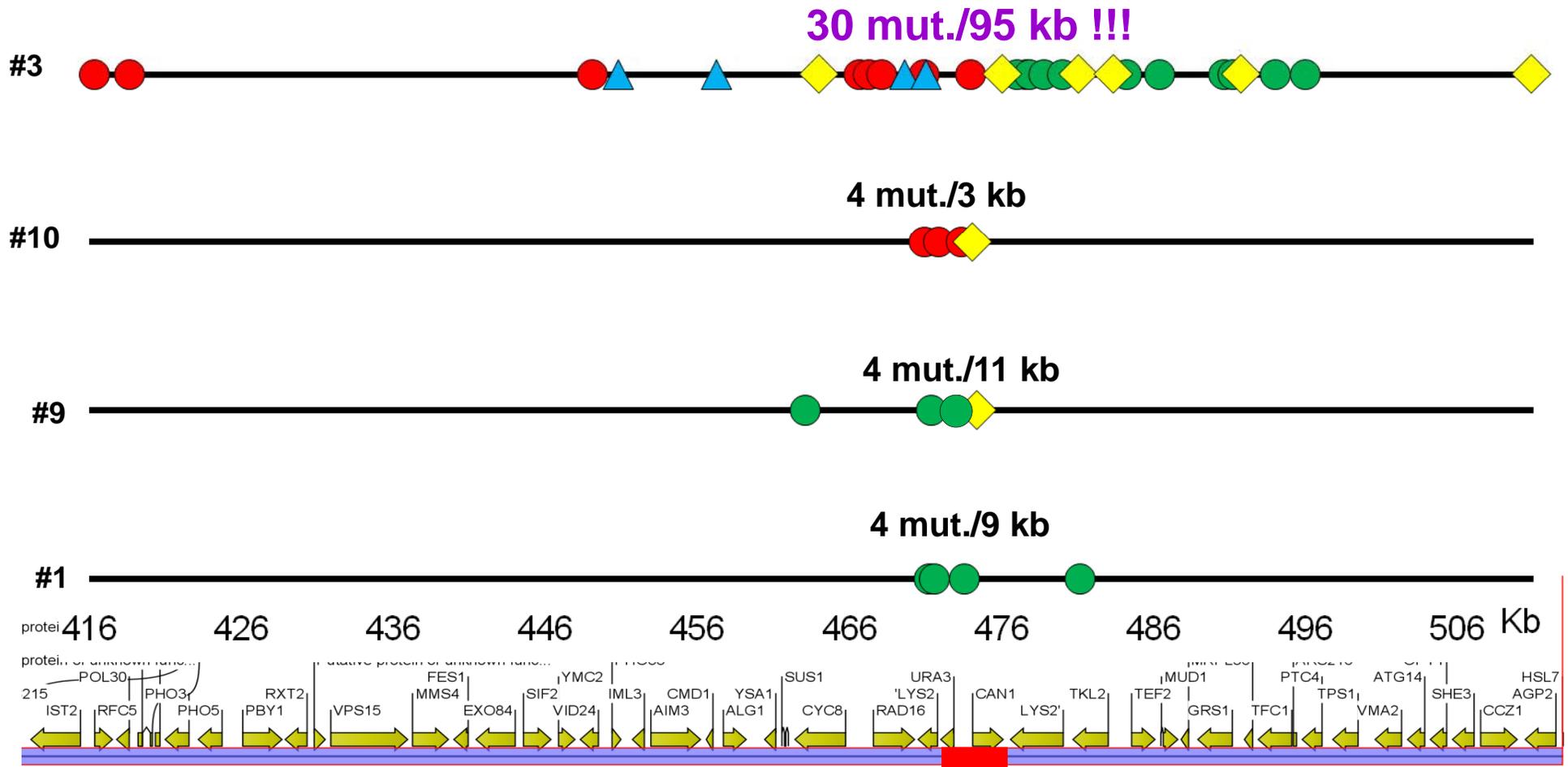
$P_{H_0} < 0.03$

How Large are Strand-Coordinated Mutation Clusters?

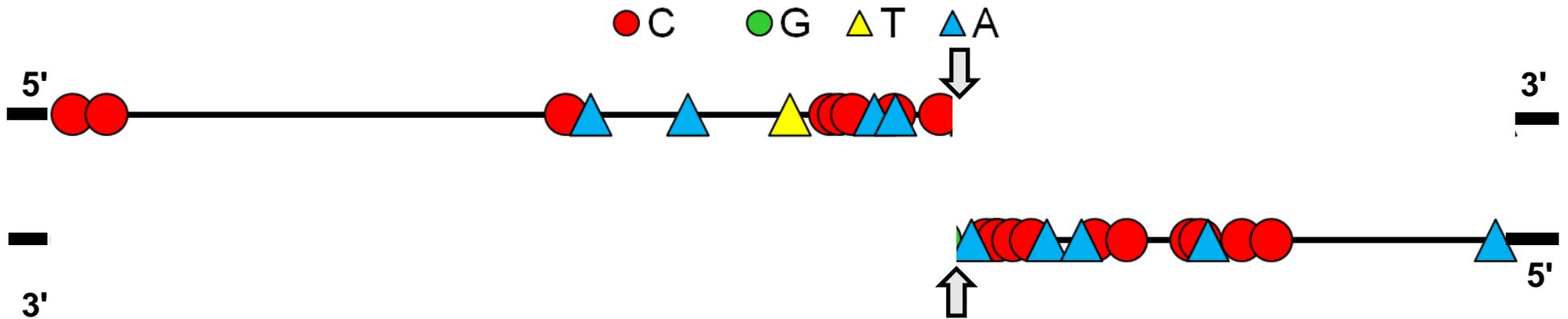
Address by whole-genome re-sequencing



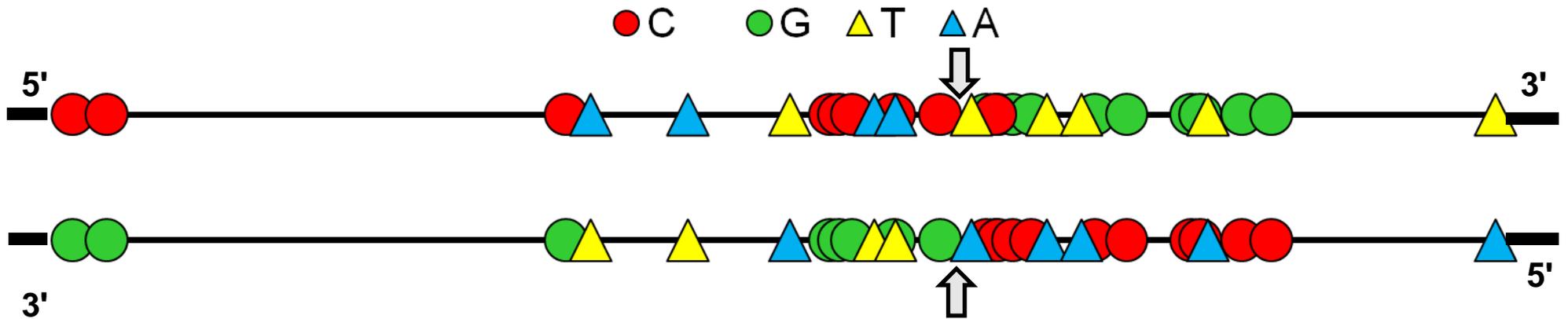
How Large are Strand-Biased Mutation Clusters?



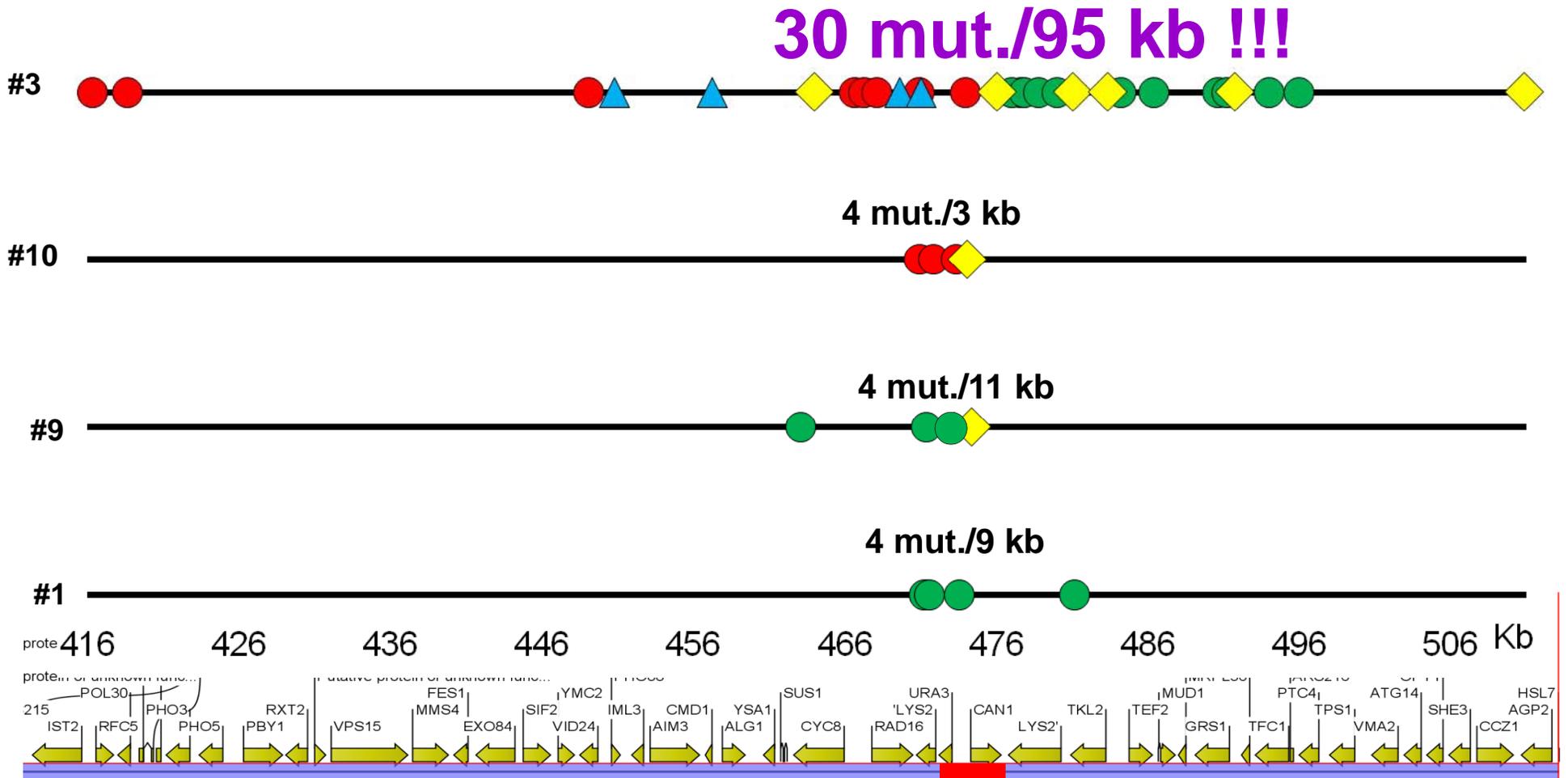
Large Cluster Can Arise from a DSB with Abnormally Long Resection



Large Cluster Can Arise from a DSB with Abnormally Long Resection

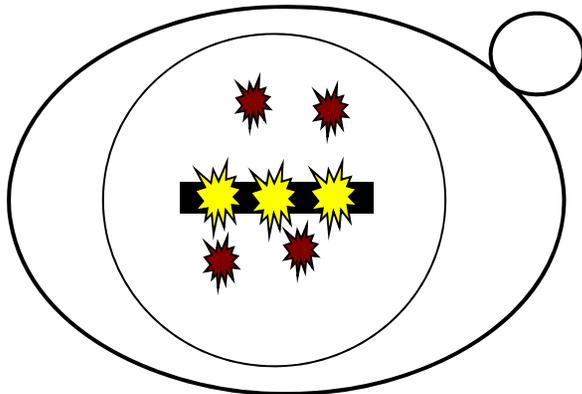


How Many Mutations in the Rest of the Genome?

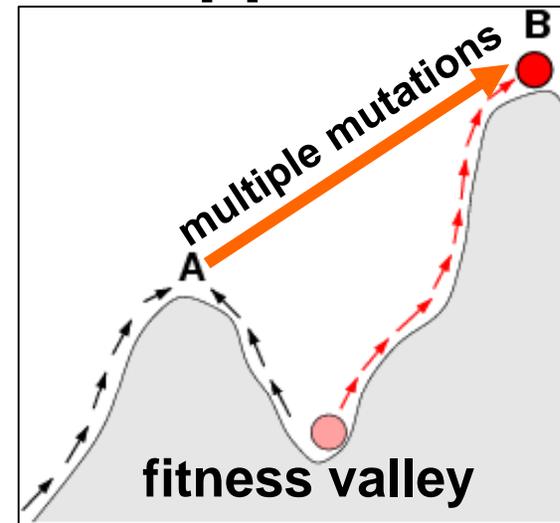


High Mutation Density in a Cluster; Low Mutation Load in the Rest of the Genome

Mutations	Clusters 3-95 kb	The rest of genomes ~1,100-1,200 kb
Number	4-30	9-14
Density	0.3-0.8 mut/kb	~0.01 mut/kb



Does it happen in nature?



Damage-Induced Localized Hypermutability

- *Finding with small reporters*
 - 1000-fold in ss DNA at DSBs and telomeres
- *Large size of a region*
 - up to 100 kb

GENOME-WIDE SCALE

- *Multiple regions in a genome*
 - simultaneous LHM in several uncapped telomeres
- *Vast mutation clusters caused by chronic mutagenesis*
 - 4-30 mutations in 3-100 kb
 - more mutations in a cluster than in the rest of the genome

Damage-Induced Localized Hypermutability -- -- Questions and Perspectives

- Sources and mechanisms
 - Acute and chronic DNA damage
 - Meiosis
 - Uncoupled replication forks
 - ?? dsDNA ?? (chromatin, transcription, etc.)
- Role in evolution and population dynamics
- Impact on human health (cancer, genetic disease)
- Detecting transient stretches of ssDNA in a cell
- **Genomic toxicology – use whole genome as a cumulative dosimeter of mutagenic insults**

Acknowledgements

Chromosome Stability Section, LMG, NIEHS

- Yong Yang
- Joan Sterling
- Francesca Storici (now at Georgia Tech.)
- Steve Roberts

- Mike Resnick

Molecular Genetics Core, NIEHS

- Nell Burch

UNC High-Throughput Sequencing Center

- Piotr Mieczkowski